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6331

Combined Effects of Drugs and Electrical Excitation of Cortical Motor Area in Cats.*

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It was shown that, following the method of François-Franck,[1] between 35 and 55 clonic convulsions could be elicited in cats by stimulating the cortical motor area with the induced tetanizing current before the animals succumbed.[2] Wortis, Coombs and Pike[3] observed that from about 5 to 10 clonic convulsions can be induced by the administration of convulsant drugs such as camphor monobromide before respiration fails and blood pressure falls to base line.[4]

In this series, which first developed as a set of controls in another group of experiments, I wished to see whether a combination of both methods would be more or less effective in prolonging or curtailing the number of convulsions and the resistance of the animal, than the use of one method alone.

Two methods of procedure were carried out.

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* The expense of the experiments reported in this paper was defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

[1] François-Franck, 1887. *Léçons sur les fonctions motrices du cerveau*, Octave Doin, Paris.

[2] Coombs, H. C., *Am. J. Physiol.*, 1932, **100**, 65.

[3] Wortis, S. B., Coombs, H. C., and Pike, F. H., *Arch. Neurol. and Psychiat.*, 1931, **26**, 156.

[4] Coombs, H. C., and Pike, F. H., *Am. J. Physiol.*, 1931, **97**, 92.

1. In a series of 10 cats, the motor area was exposed under ether, and the anesthetic then intermitted. After recovery from ether, the cortex was stimulated with a mild induced tetanizing current as previously described. When the cortex had been stimulated from 25 to 40 times, with the usual clonic convulsions, a dose of camphor monobromide which corresponded to about the estimated minimal convulsive dose for the weight of the animal, was injected intravenously. In most cases, it proved fatal. Respiration failed and the animal usually died in tonic extension without the usual typical clonic convulsion, or with only a few clonic twitches.

2. In another series of 12 cats, after exposure of the motor area under anesthesia, with one electrical excitation to make sure the clonic convulsive response was readily elicitable, the estimated minimal convulsive dose of camphor monobromide or absinth was administered. When the convulsion due to the drug had worn off, the motor cortex was stimulated as before, until the animal succumbed, and it was found that the number of electrical excitations which could be done under these conditions had a range of from 10 to 20, with an average between 14 and 15—obviously far below the range of 35 to 55 convulsions which could be elicited in a similar way when no drug was given. Moreover, following a clonic convulsion elicited by a minimal dose of the convulsant drug, the response to electrical excitation was stronger than when electrical excitation only was done, the clonic convulsion sometimes lasting from 45 seconds to one minute, with clonic twitches persisting for another minute after the convulsion had ceased.

It has been shown in the case of convulsant drugs and cerebral anemia that when combined, the animal succumbs to a sum of less than half of each agent which would be fatal if used alone (Coombs and Pike[5]), that is, excitation from 2 different chemical sources appears to be more destructive to the cells of the central nervous system than a considerably greater amount of excitation from only one source. This appears to be the case also with excitation from a physical and a chemical source, *i. e.,* electrical excitation and camphor. Under these conditions, animals succumb to about one-third the number of electrical excitations plus about one-tenth the amount of the drug which, when taken separately, has been found to be lethal in a large number of cats.

In connection with work by Pike and Notkin[6] on the summation of the effects of drugs, I may call attention to the following considerations. We have no measure of the work done either by the chemical agent or the physical agent in eliciting a convulsion. The mechanism of stimulation by chemical agents must differ in some essential respect from that of stimulation by physical agents since the sum of the 2 effects, *i. e.*, excitation by a chemical agent first, and then by a physical agent, or conversely, excitation by a physical agent first and then by a chemical agent, differs so widely from the total effect of either chemical or physical excitation alone. From the numerical ratio one might say that one chemical excitation will do about as much damage as several physical excitations and that the end result of the processes set up by either form of excitation renders the cells more vulnerable to the end result of processes set up by the other.

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[5] Coombs, H. C., and Pike, F. H., *Am. J. Physiol.,* 1932, **99**, 521.

[6] Notkin, J., and Pike, F. H., *Am. J. Psychiat.,* 1931, **10**, 771.

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Buffer Influence Upon Response of Striated Muscle to Caffeine Stimulation in Fatigue Studies.

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The experiments cited here indicate the influence of the buffered solvent upon the response of the gastrocnemius muscle of *Rana pipiens* Schreber with respect to caffeine action. In recognition of the importance of the pH factor upon physiological phenomena, this environmental variant was controlled at a constant value. It was noted. however, in studying the effect upon neuro-muscular responses in fatigue studies, that in spite of the constant pH conditions as maintained at 7.3, which is the pH of frog blood and lymph, definitely variable results were obtained. Even the complete reversal of the characteristic responses was observed in accordance with the nature of the buffer employed as the particular injection medium for the substance concerned.

The influence of ion variation on the action of different concentrations of Ca and K in a Locke solution medium upon smooth muscle action, as shown by Salant and Parkins,[1] demonstrates that the effect of an alkaloid—in their experiments ergotamine was used—varies with the pH and with the nature of the ion. There is also a definite relationship of the observed effect to the inter-relationship of both the factors concerned. In the experimental series here presented, the influence of the medium is noted to be effective also upon the striated muscle response, independent of the pH. The constant pH value with a variation in the nature of the ions employed for that purpose demonstrated the divergent effect of caffeine upon striated muscle in the presence of different buffer media.

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[1] Salant, Wm., and Parkins, Wm. M., *J. Pharm. and Exp. Therap.*, 1932, **45**, 315.

Caffeine effects have been reported by several investigators. In low concentrations, Bock[2] found that caffeine increases the extent of the contraction record for striated frog muscle; but, in high concentrations, Hartree and Hill[3] noted contracture and rigor with an accompanying loss of excitability. Cheney[4] has demonstrated that caffeine improves the uniformity of the crest wave of fatigue curves of the gastrocnemius muscle—sciatic nerve preparation and has shown[5] that the caffeine treatment of pithed frogs by a dorsal lymph sac injection followed by a 25-minute absorption period prior to stimulation, increases slightly both the height of contraction and the time to fatigue. In addition, Cheney[6] determined the optimum caffeine dosage for the frog *(Rana pipiens* Schreber) to be 0.1 mg. per gm. of body weight.

The mechanical apparatus was essentially the same as described by Cheney[5] for the pithed frog, isotonic contractions. The dosage in all instances, however, was the optimum dosage referred to above. In cases in which the medium was injected without the caffeine, a volume was used equivalent to the quantity representing the amount of injection required to give the optimum dosage (0.1 mg./gm. body weight) if caffeine had been involved. The absorption time from the dorsal lymph sac was constant: a 30 minute period. Other constants included the strength and rate of stimulation (1 per 5 seconds), and the pH of the injection medium; *i. e.,* 7.3, except in the distilled water and aqueous caffeine, which was 6.7 as determined by the electrometric method. A uniform rate of rotation was maintained with respect to the recording drum on which 1 cm. of graph surface registered 22 contractions. The after-load in each instance was equal to the body weight of the particular animal utilized. Moreover, the gastrocnemius record of the untreated limb was always compared directly with the caffeine-treated gastrocnemius of the *same* animal. One-half of the data was derived from the use of the right muscle as the non-treated record, and the remaining half employed the left gastrocnemius, in order to eliminate any possible difference in the paired muscles of the same animal.

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[2] Bock, J., *Heffter's Handb. d. exp. Pharm.,* 1920, **2**, 508.

[3] Hartree, W., and Hill, A. V., *J. Physiol.,* 1924, **58**, 441.

[4] Cheney, R. H., *J. Pharm. and Exp. Therap.,* 1931, **43**, 457.

[5] Cheney, R. H., *Arch. Intern. d. Pharm. et d. Therap.,* 1932, **42**, Fasc. II, 173.

[6] Cheney, R. H., *J. Pharm. and Exp. Therap.,* 1932, **45**, 389.

The pH constant was maintained by various buffers which showed a difference in the effect on muscular fatigue in accordance with the buffer composition. The statistical basis of comparison for the different series was the Xy value (Time to one-half fatigue x Height of Contraction at that time) in frogs of miscellaneous body weights; and also, the percentage increment or decrement of B/A, where "B" represents the caffeine-treated muscle record of its Graph Area, and "A" refers to the non-treated muscle. Ten or more experiments were performed in each of the series listed below.

TABLE I.

Series	Preparation	Difference in XY value between A and B.	Aver. % Increment or Decrement of B/A Graph Area.
I	Non-treated Right Gastrocnemius "A"	0.5	4
	Non-treated Left Gastrocnemius "B"		
II	Non-treated Muscle "A"		
	Distilled water-treated "B"	10.62	26
III	Non-treated Muscle "A"		
	Aqueous caffeine-treated "B"	11.76	30
IV	Non-treated Muscle "A"		
	Bicarbonate buffer Ringer-treated "B"	13.4	20
V	Non-treated Muscle "A"		
	Bicarbonate buffer Ringer-caffeine "B"	15.55	33
VI	Non-treated Muscle "A"		
	Phosphate buffer-treated "B"	11.32	26
VII	Non-treated Muscle "A"		
	Phosphate buffer caffeine-treated "B"	—4.69	13
VIII	Non-treated Muscle "A"		
	Phosphate buffer Ringer-treated "B"	7.43	13
IX	Non-treated Muscle "A"		
	Phosphate buffer Ringer caffeine-treated "B"	—3.55	8

It will be noted that the Xy values of the right and left non-treated muscles are approximately equal regardless of which leg was fatigued first. A 50% alternation of the limb treated was carried out routinely to avoid any possible error. It is clear also that the medium alone so affects the osmotic conditions, even in the case of frog Ringer's solution, that some positively stimulatory effect is produced. Such an effect is, however, always less than the results recorded *after* the addition of the optimum dosage of caffeine. A reversal of the preceding values occurs for the caffeine effect in the presence of a phosphate buffer.

Such a reversal of the effect of a substance with a change in the buffered solvent, and especially with phosphate buffers, has been observed also in blood studies as well as in these muscle investigations. In some way the phosphate ion seems to prevent the caffeine from entering the tissue or of acting upon the tissue with its characteristic effect. It is highly improbable that there is any significant reaction between the caffeine and the phosphate since the caffeine is a very weak base. A reaction with the formation of such a compound as caffeine phosphate would not occur, at least until the pH was far more alkaline than 7.3, which is the figure with reference to frog blood and lymph, and therefore, this value was maintained throughout these experiments.

Summary. 1. The characteristic positive effect of caffeine in delaying the rapidity of fatigue in the gastrocnemius muscle is apparent when administered in an aqueous medium, or in a bicarbonate buffered-Ringer medium. 2. The effect of the medium containing the caffeine is greater than the effect of the medium alone. 3. A reversal of the usual caffeine effect upon the fatigue record of striated muscle occurs when the caffeine is introduced in a phosphate buffered medium, or in a phosphate buffered-Ringer medium. 4. Both phosphate-buffered caffeine solutions cause a negative effect to that of the medium alone. 5. The necessity of discretion in the choice of a buffer in the maintenance of the desired pH of a solvent medium is evident; and, the elimination of an aqueous or Ringer phosphate buffered solution as a solvent for caffeine, in studies of the effect of caffeine upon the fatigue record of the striated (gastrocnemius) muscle, is indicated specifically.

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Occurrence of Rough Pneumococci in Lungs of Patients with Lobar Pneumonia.

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"S" and "R" forms of pneumococci are considered, in a general way, to represent respectively virulent and avirulent phases of the organism. It seemed reasonable to suspect that degradation from

"S" to "R" forms might take place in the mechanism of recovery from pneumococcus infection in living tissues. Wadsworth and Sickles[1] recovered attenuated pneumococci from the blood stream and heart valves of horses previously immunized to pneumococci. Reimann[2, 3] demonstrated the very occasional occurrence of "R" forms in the sputum of patients with lobar pneumonia. Paul[4] produced "R" forms *in vivo* under certain experimental conditions in dogs.

In the present communication is reported the recovery, by direct lung puncture, of "R" variants of the pneumococcus from the lungs of a majority of lobar pneumonia patients studied. Clear cut cases only of lobar pneumonia were studied. Punctures were made at points of maximum consolidation. Lung puncture, in pneumonia, has been shown to be a safe procedure.[5] Material obtained by lung puncture was cultured at once in glucose broth and upon blood agar plates. For the latter, following the work of Sia and Chung,[6] 5% dog blood was used to facilitate recognition of "R" and "S" forms. Colony studies were made after 18-22 hours incubation, longer incubation being used only when growth was retarded. Smears of the lung puncture material were studied with Gram's and Hiss capsule stains. In several cases portions of the puncture specimen were inoculated intraperitoneally into white mice.

Identification of organisms was based upon cultural and microscopic characteristics, and in most cases upon bile solubility and type specific agglutination. In the majority of cases of typical lobar pneumonia, pure cultures were obtained upon lung puncture. Occasionally the pneumococcus was accompanied by *H. influenzae*. This interesting finding is being studied further.

Colonies were examined under the Zeiss colonyscope with oblique illumination. Colonies classed as "R" showed variations in their borders and surfaces from gross irregularity to fine grayish stippling; they were indented and firm in character and moved as a solid mass. "S" colonies presented clear, glistening, rounded, highly refractile surfaces and were quite soft.

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[1] Wadsworth, A. V., and Sickles, G. M., *J. Exp. Med.*, 1927, **45**, 787.

[2] Reimann, H. A., *J. Exp. Med.*, 1925, **41**, 587.

[3] Reimann, H. A., *J. Exp. Med.*, 1927, **45**, 807.

[4] Paul, J. R., *J. Exp. Med.*, 1927, **46**, 807.

[5] Rosenow, E. C., *J. Inf. Dis.*, 1911, **8**, 500. Dochez, A. R., personal communication. Thomas, H. M., and Parker, F., *Arch. Int. Med.*, 1920, **26**, 125. Glynn, E., and Digby, L., Medical Research Council Special Report No. 79, 1923.

[6] Sia, R. H. P., and Chung, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 245.

In Table I, colonies are classified as "R+", "R", and "S" to differentiate between complete rough and less rough or intermediate forms. This variation is of considerable interest. For the discussion below, however, the use of "R" and "S" suffices. Of 24 lung punctures, 11 were done during life and 13 immediately after death. Positive cultures of pneumococci were obtained in all but 4 cases, each of which was in crisis or lysis at the time of puncture. Slight broth growth was obtained once after crisis (Case No. 7) but could not be subcultured. There were 16 successful colony

TABLE I.

			Stained Smears		Dog Blood Agar Plates							
No.	Day of disease	Type	Pneumococci	Capsules	Hr. growth	Amt. growth	R+	R	S	+ Broth growth	R = recovered; D = died	Remarks
1	24	II	+	—	48	++		+	+	+	D	*H. Influenzae(?)*
2	6	II	+	+		0				++	D	
3	6	II	+	+	18	++		+	+	++	D	Staphylococcus, *H. Influenzae*
4	4	II	+	+	24	++	+	+	+	++	D	*H. Influenzae*
5	8	gIV	0	0		0				0	D	Acute pericarditis. Pneumonia resolving
*6a	8	II	+	+	20	+			+	+++	—	
6b	9	II	+	+	22	++		+++	++	++	D	*H. Influenzae(?)*
*7	6	I	0	0		0				+	R	Cultured after crisis
*8a	4	II	+	+	18	++			+	+++	—	
8b	8	II	+	+	48	+		++	+	+++	D	S colonies virulent; R, avirulent
*9	11	gIV	0	0		0				0	R	Cultured during lysis
*10	7	II	+	—	48	+			±	+	R	Massive empyema
*11a	3	VII	+	—	24	+	++			++	—	Peculiarly "tufted" R colonies
11b	4	VII	+	—	18	+	++	+		++	D	
12	8±	II	+	+	40	+	+	+	+	++	D	
13	11	VIII	+	+	20	+		+	+	+	D	Confluent Bronchopneumonia
*14	7	gIV	±	—		±				0	R	Cultured 24 hrs. after crisis
15	6	II	+	+	20	++			+	+	D	Cultured at autopsy. Mixed growth
16	3	II	+	+	48	+	+	+	+	++	D	
*17	3	gIV	+	0		0				0	R	Cultured during crisis
*18	2	II	+	+	24	++	+		++	+++	D	Mixed growth. *H. Influenzae*
19	5	II	+	+		0				++	D	
*20a	3	II	+	0	22	+		+		++	R	Pnc. of low virulence. Restored by mouse passage
*20b	4	II	+	—	20	++	+	+		++	R	

* Studies from living patients.

studies. In 10 done post mortem "R" colonies occurred alone once, "S" colonies alone once, and "R" and "S" 8 times. Six successful colony studies from living patients showed "R" colonies alone in 3 instances, with one subsequent death; "S" alone in 2 with 2 deaths; and "R" and "S" in one which resulted in death. In the total series "R" colonies were recovered 13 times and "S" colonies 12.

Case No. 20 is of particular interest in that pure cultures growing variant "R" colonies were isolated twice during life. The first puncture was obtained at the height of the disease, the second at the onset of crisis. Colonies obtained from the latter were more nearly completely rough than those obtained from the former. Both strains showed very low virulence for mice and inagglutinability in type specific serum, but were restored to virulence and agglutinability by mouse passage.

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Effect of Anterior Pituitary Upon Production of Red Pigment in the Salamander Pseudotriton ruber ruber (Soninni).

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A marked change in coloration occurs at the time of metamorphosis in most salamanders. We have obtained evidence that this change is not produced by the thyroid hormone in the case of red pigment formation. Thyroidectomized larvae of *Hemidactylium scutatum* will assume the red coloration of the adult although they do not metamorphose.[1] Larvae of the red salamander *Pseudotriton ruber* when precociously metamorphosed with thyroid extracts are yellow and not red. Since the anterior pituitary is known to play a part in normal metamorphosis we have tested the effect of anterior pituitary implants upon red salamanders which have been precociously metamorphosed with thyroid solutions.

Fifty larvae of *Pseudotriton ruber,* 30 to 50 mm. in length, were metamorphosed in a solution of 1:15,000 desiccated thyroid. Before transformation was completed 7 of the largest specimens of the same size (Group A) were selected and each of 4 given subcutaneous implants of 2 pars anterior of toad pituitary. Three specimens received muscle, to serve as controls. The gland material was taken from freshly killed adult *Bufo fowlerii,* and given at regular intervals, 3 times a week. The salamanders, throughout the experiment, were kept without food, in separate dishes, at room temperature, with just enough spring water in the dish to keep the surfaces moist.

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[1] Noble, G. K., and Richards, L. B., *Anat. Rec.*, 1931, **48**, 58.

A few days later 5 fully metamorphosed salamanders of the series (Group B) were implanted in the same manner, 3 serving as experimental, 2 as control animals. At the same time 9 more specimens (Group C), also completely transformed, were implanted, but on a different schedule. The 5 experimentals were implanted twice a week, with 3 pars anterior each, the 4 controls with muscle. A fourth group (D), of 4 specimens, completely transformed, received implants of pars intermedia plus pars posterior. Two salamanders were implanted twice a week, with 4 pituitaries each, and 2 implanted 3 times a week with 3 pituitaries each. After 30 days, or 12 grafts for Groups A and B, and 9 for Group C, the implanting was stopped. The reaction to the anterior pituitary was apparent in all of the experimental specimens but most marked in Group A, on which the implanting was begun before the completion of metamorphosis. After the 6th implant all 4 experimental animals assumed a reddish tinge not seen in the controls. Their color became increasingly red with every implant. After the 12th implant they ranged from a capucine-yellow to flame-scarlet.[2] The control salamanders changed color slightly, as did the stock from which all were taken, becoming lighter in hue—and finally ranged from orange-buff to antimony yellow. In Group B, on which the implants were begun after the completion of metamorphosis, but in the same manner as Group A, the results are positive but not as marked. The 3 experimentals, range from capucine-yellow to orange-chrome, not achieving the flame-scarlet of Group A. The 2 controls were light-orange-yellow. In Group C (implanted twice a week) the color of the experimentals, after 12 implants, was only slightly more red than that of the control specimens. The 5 salamanders receiving anterior lobe ranged from ochraceous-buff to a shade between capucine-yellow and orange. The 3 controls ranged from light-orange-yellow to buff-yellow. Group D, implanted with pars intermedia plus pars posterior, became very dark, due to the expansion of the melanophores, and assumed a greenish hue. The black pigment overshadowed the background color to such an extent that it was practically impossible to name that color. The general effect was of olive-lake to yellowish-citrine. From these data it is evident that the red color of an adult *Pseudotriton ruber* may be induced in prematurely metamorphosed individuals by anterior pituitary implants. It seems probable that in the normal development of the salamander it is the release of a quantity of anterior pituitary hormone, initiating metamorphosis, which brings about the assumption of adult red color at this time.

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[2] Ridgway, R., 1912, Color Standards and Nomenclature, Washington.

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Typhoid Agglutinins as Influenced by the Conditioned Reflex in Man.

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In order to throw some light on the relation of the nervous system to the production of antibodies, an investigation of the artificial production of typhoid agglutinins in man was undertaken. Following the procedure which Metalnikov[1] used with animals, the plan followed in 3 experiments was to inject patients for 21 successive days with a small dose of typhoid vaccine subcutaneously, immediately after application of an ice cube to the cheek. After a rest period of approximately 2 weeks the patients were given the conditioning stimuli alone, that is, ice cube, and the prick of the needle. The curve of typhoid agglutinins was determined.

In the first experiment with 12 subjects, 6 received typhoid vaccine daily and 6 received injections of sterile saline. The latter served as a control group and did not show any titer during the injection period. Following the administration of conditioning stimuli alone to the first 6 subjects, 2 showed a slight rise in titer from 1-200 to 1-250.

In the second experiment 12 subjects received typhoid vaccine (groups of 4 receiving different dosages). Two weeks later the conditioning stimuli alone were applied. Blood was taken 75 minutes later. Of the 11 subjects so tested 6 showed a rise in titer of one tube (one dilution-step), one a rise of 2 tubes and one a rise in 3 tubes, in the usual dilutions of 1-20, 1-40, 1-80, 1-160, 1-320, etc. Three who had responded feebly to the vaccine showed no change. Twenty-four hours later the blood examination showed a marked decrease in titer in all subjects who had previously shown a rise. Subsequent conditioning stimuli again led to a rise.

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[1] Metalnikov, S., *Ann. d. I. Inst. Past.*, 1931, **46**, 137.

In a third experiment, 16 subjects were given the same dosage of typhoid vaccine. The conditioning stimuli were not applied until 19 days after the administration of vaccine had been discontinued. Two of the subjects received no further injections and were utilized as controls. Eight subjects received conditioning stimuli alone; 3 received typhoid vaccine in a non-conditioned setting; and 3 received typhoid vaccine with conditioning.

The titers were read 3 times with a 6X magnifier by each of 3 observers, none of whom knew the classification of the subjects. The 2 control subjects showed no rise in titer. Six of the 8 subjects receiving conditioning stimuli alone gave a rise in titer of one tube, and 2 gave a rise in titer of a half tube. Two of the 3 subjects receiving vaccine in a non-conditioned setting showed a rise in titer of 1½ tubes, the third showing no rise. Two of the 3 subjects receiving vaccine with conditioning showed a rise of one-half tube, a third showed a rise of one tube.

Dr. Irving Lorge of Teachers College very generously went over all our data and according to his findings, based on the technic devised by Fisher, in which the 3 observers were differently weighted, there was a rise in titer in subjects receiving conditioning stimuli as compared with their own titers before the stimuli were applied.

It is doubtful from our data that a significant rise in typhoid agglutinins follows conditioning stimuli applied to suitably prepared subjects.

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Mechanism of Bacteriophage Action in Staphylococcus Bacteremia.*

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Four young rabbits, 1100 to 1320 gm. in weight, were inoculated intravenously with a suspension of staphylococcus which had been isolated from human bacteremia. Each animal received an intra-

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venous injection of 2 cc., equivalent to 5400 million living staphylococci. One rabbit was sacrificed 155 minutes after inoculation. Sections of spleen and liver revealed phagocyted leucocytes and partly disintegrated nuclear granules. Only one pair of cocci was recognized within a Kupffer cell after considerable search. A second animal was sacrificed 238 minutes after inoculation. Sections of the spleen revealed many phagocyted granules and nuclear fragments but no definite bacteria. In the liver, however, there were distinct and definite cocci within the somewhat swollen Kupffer cells. The third rabbit received an intravenous injection of 2 cc. undiluted asparagin staphylococcus bacteriophage 103 minutes after the bacterial inoculation and was sacrificed 131 minutes later, or 234 minutes after the bacterial inoculation. Sections of the spleen of this animal showed numerous phagocyted nuclear granules in the marginal zones about the splenic follicles and along with them poorly differentiated cocci in small numbers. In sections of the liver the phagocyted cocci were very abundant and distinctly differentiated, decidedly more abundant than in the liver of the second animal. The fourth rabbit received a similar dose of bacteriophage 106 minutes after bacterial inoculation and was sacrificed 89 minutes later or 195 minutes after the bacterial inoculation. Sections of the spleen of this animal contained unmistakable groups of micrococci within the reticular cells about the follicles and in some locations these cocci were numerous. In the liver also, the Kupffer cells contained abundant phagocyted cocci. The observations indicate that one immediate effect of intravenous injection of bacteriophage in bacteremia is to favor more rapid phagocytosis of the bacteria which are circulating in the blood.

Another lot of 4 young rabbits, weighing 300 to 400 gm. received similar intravenous inoculation with another septicemic strain of staphylococcus. The dose was 2 cc. of a suspension equivalent to 3500 million living cocci. One animal was sacrificed 203 minutes after inoculation. The blood culture taken at death showed 119,400 viable bacteria per cc. of blood, representing only about one-thousandth part of the bacteria introduced into the blood 203 minutes before. Sections of the spleen and liver revealed phagocyted cocci and also phagocyted red blood cells and leucocytes, especially conspicuous in the Kupffer cells. A second rabbit in this group received 2 cc. of bacteriophage intravenously 130 minutes after the bacterial inoculation and 110 minutes later, or just 4 hours after inoculation with bacteria, this animal was sacrificed. The blood culture at death showed 67,700 viable cocci per cc., slightly more than half the number found in the first animal. The sections of spleen and liver contained somewhat more phagocyted cocci and there was less evidence of phagocytosis of red and white blood cells. The third animal in this group died 21 hours after inoculation. The blood culture, at necropsy, showed 15,800 viable cocci per cc. of blood or approximately one-eighth the number found in the first animal, which was killed some 17 hours earlier. The spleen and liver contained moderately numerous phagocyted cocci and there were many phagocyted leucocytes in the liver. In the kidney there were many large colonies of staphylococci in the medulla, for the most part plugging the capillary blood vessels in this region. The fourth animal in this group received 2 cc. of bacteriophage intravenously 140 minutes after the bacterial inoculation. The next day it appeared to be in good condition. It was sacrificed 20 hours 45 minutes after the injection of bacteriophage and 23 hours 5 minutes after inoculation with the bacteria. The blood culture at death showed 10 viable cocci per cc. or less than one-thousandth of the number found in the third animal 2 hours earlier. Microscopic study of sections of spleen, liver, kidney and lung failed to disclose any recognizable phagocyted bacteria. Structural alterations in the organs will not be discussed here. The observations on this second group of 4 animals are in accord with those on the first group, indicating that one effect of the injected bacteriophage is the promotion of more rapid phagocytosis of the bacteria by the endothelial cells. There is, however, an additional disclosure, namely that the presence of the bacteriophage tends to restrain the further growth of the bacteria which have lodged in the internal organs and also favors the more rapid and efficient intracellular digestion of the phagocyted bacteria.

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Experimental Infarction of the Interventricular Septum of the Heart in the Dog.

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Infarction of the interventricular septum of the dog's heart by ligation of the front septal branch of the left coronary artery was successfully produced in 4 of 28 animals. The changes involved from one to two-thirds of the surface of the left side of the septum, and about one-tenth of the right. The infarct was widest about 1 cm. below the attached margin of the aortic cusps, its edges jagged, though well defined. and tapering in the direction of the apex. In one heart, 10 days after operation, the course of the left branch of the bundle of His was plainly visible just beneath the endocardium and over the infarcted muscle. Roughening or other alteration of the lining of the cavities of the heart was not observed.

Microscopically the tissues immediately beneath the endocardium, including the Purkinje fibers of the bundle, were unaltered, but the subjacent muscle had undergone coagulation necrosis and was in varying stages of organization, according to the age of the lesion. Approximately one-tenth of the A-V node was involved in the infarction, regions of varying size disseminated irregularly throughout the node.

There was no auriculo-ventricular dissociation demonstrated in post-operative electrocardiograms, and no significant irregularity in rhythm noted by other methods. The earliest after operation that an initial post-operative electrocardiographic tracing was made was 2 days, the latest, 10 days; a transient effect on the rhythm may have escaped recognition.

It seems probable that obstruction of the septal branch of the left coronary artery in the dog does not produce permanent dysfunction of the A-V node or the His bundle. The endocardium and tissues immediately beneath it, including part of the conduction system, may have received nourishment by seepage of blood in the same direct way that the inner lining of some of the arteries is nourished. It is possible that if the animals were kept alive over a period of several months, scar formation and contraction would damage the conduction system sufficiently to produce lasting heart block.

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A noteworthy observation was the absence of any thrombosis of the lining of the cavities of the hearts of these dogs, a state of affairs in marked contrast with that in the human heart where mural thrombosis is a frequent sequence of interference with the coronary circulation.

Further similar experiments are in progress.

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Effects of Pituitary Antidiuresis on Non-Cardiac Edema.

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From a previous study, describing the induction of pituitary antidiuresis in non-edematous subjects,[1] we were impressed by the magnitude of the net loss in body weight at the end of the recovery period. The obvious fact, that the reduction in weight which follows withdrawal of the antidiuretic agent is due under these conditions to loss of body water with its accompanying mineral elements, suggested the desirability of determining the effects of the procedure on the water and mineral exchanges in the presence of edema.

This has been done in 2 cases of typical lipoid nephrosis, one of chronic glomerulonephritis with superimposed nephrosis and one of edema of undetermined etiology. The total serum proteins were low in these subjects at the time of the tests. The case of edema of undetermined cause differed from the other 3 in showing no proteinuria and no increase in the blood lipoids. Throughout the entire period of observation all 4 patients were maintained in the metabolism ward under practically uniform conditions as regards room temperature and humidity, diet and routine for obtaining the body weight and for collecting samples of the excreta. The total water exchange was determined according to the method outlined by Newburg and Johnston.[2] After a preliminary control period of several days on a standard diet relatively low in NaCl, the patient was given a medium-sized dose of the antidiuretic pituitary extract (pitressin P. D.) subcutaneously at 3-hourly intervals for one or 2 days, the exact body weight being measured every 6 hours.

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[1] McQuarrie, Irvine and Peeler, D. B., *J. Clin. Invest.*, 1931, **10**, 915.

[2] Newburg, L. H., and Johnston, M. H., *J. Clin. Invest.*, 1930, **8**, 161.

After this had been increased by from 3 to 5% due to water storage, the pitressin was discontinued. Balances for the following mineral elements were determined: Na, K, Ca, Mg, Cl, N, S and P. The entire procedure was carried out on 2 occasions for each patient.

The results may be briefly summarized as follows: All 4 of the patients showed a visible increase in the intensity of the edema at the end of the period of antidiuresis. That the water thus forcibly retained was temporarily stored in the extracellular reservoirs of the body, and not within the cells, is indicated by the fact that the chief mineral elements finally excreted with it were Na and Cl. The response of the patient with chronic glomerulo-nephritis differed sharply on both occasions from the other subjects studied and from the normal by the fact that her weight level at the end of the test was no lower than that of the fore period. Whereas the normal subject usually loses considerable amounts of Na and Cl during and immediately following the period of antidiuresis (Manchester[3]), this patient showed no increase in the elimination of these or any of the other inorganic elements. In contrast to this response, the 2 patients with primary nephrosis showed strongly negative NaCl balances and finally corresponding losses of body water. In the younger of the 2 there was on 2 successive occasions a definite "escape" from the antidiuretic effect of the extract before the gain in weight had reached the height usually attained. The diuresis which followed was continuous over a period of several days on the first occasion, until the edema had almost completely disappeared. The weight was decreased from 20.2 to 16.6 kg. in 5 days. Repetition of the procedure shortly after the weight had again become stationary at a lower level resulted in a still further reduction. The response of the patient with edema of undetermined etiology was intermediate between that of the case of chronic glomerulonephritis and that of the 2 cases of nephrosis. The net loss of body water in this patient was roughly proportional to the excess of Na and Cl excreted as in the other cases. Aside from this effect on the Na and Cl balances and the flushing out of a small amount of extra K during the period of recovery diuresis, no significant disturbance in the mineral exchange was observed in any of the patients studied. Except for a fairly marked increase in the albumin and a simultaneous decrease in the globulin fraction in one case of nephrosis, the serum proteins were essentially unchanged. The lecethin and cholesterol of the plasma remained unaffected. Since no ill effects result from the procedure as carried out here, further studies are being made to determine the limitations of its usefulness in initiating diuresis in cases of edema which have ceased to respond to dietary and other ordinary therapeutic measures.

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[3] Manchester, R. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 717.

6339

The Large Q Wave in Lead III of the Electrocardiogram.

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Although arteriosclerotic heart disease is not uncommon in the negro, angina pectoris is rare and coronary thrombosis much rarer. In view of the recent interest in large Q waves in lead III of the electrocardiogram as evidence of coronary artery disease, particularly coronary thrombosis, it was thought that a comparison of whites and negroes might be of interest.

Electrocardiograms which fulfilled the criteria given by Pardee[1] for large Q in lead III were selected; 192 electrocardiograms from 5280 patients, of whom approximately 4830 had heart disease. Of these 192, 143 were from whites and 49 from negroes, an incidence of 3.8% and 2.3% respectively.

In the white group with large Q in lead III, 46% gave a history of cardiac pain; 49.6% reported no pain, while 4.2% were doubtful. In the negro group, 30.6% gave a history of pain; 61.2%, no pain; 8.2% doubtful. Conversely among 162 white patients with cardiac pain, the incidence of large Q in lead III was over 12%, while among 89 negroes with heart pain, the incidence was nearly 4.5%.

Of 60 patients with a definite clinical picture of coronary thrombosis, 23, or 38% presented a large Q wave in lead III.

That arteriosclerotic heart disease is the chief cause of the large Q is shown by the following figures. A study of about one-third of all the patients indicated the following percentages of clinical diagnoses in the various etiological groups. White patients: arteriosclerotic 26.9%; hypertensive 13.6%; syphilitic 8.7%; rheumatic 12.7%; other, including unknowns and normals, 38.1%. Negro patients: arteriosclerotic 13.1%; hypertensive 23.6%; syphilitic 34.1%; rheumatic 9.8%; other 19.4%. In contrast to this, the various etiological factors contributed large Q waves in the following proportions: Whites: arteriosclerotic 41.3%; hypertensive 36.4%; syphilitic 4.9%; rheumatic 2.8%; other 14.7%. Negroes: arteriosclerotic 8.2%; hypertensive 32.7%; syphilitic 32.7%; rheumatic 6.1%; other 20.3%. Among white patients, the arteriosclerotic-hypertensive group constituted 40.5% of the whole and contributed 77.7% of the large Q waves. Among the negroes, this group was 36.7% of the whole, and contributed 40.9% of the large Q waves found among negroes.

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[1] Pardee, H. E. B., *Arch. Int. Med.*, 1930, **46**, 470.

Among 150 patients without evidence of heart disease, 3, or 2%, showed a large Q wave in lead III, but two of these Q waves were not typical and were barely included on the basis of the criteria given by Pardee.

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Physiological Effects of Injections of Various Benzene and Furan Derivatives into the Cockroach.

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This is a report of experiments upon the physiological effects of injections of various benzene and furan derivatives into the cockroach, *Periplaneta orientalis* (Linn.). Some of these compounds have been used in spray[1, 2] and vapor[3] form upon certain insects but nothing is known of the effects of injections of the substances into the roach. Injection studies on insects have been largely confined to larvae.[4, 5] In these experiments, large nymphs and a few adults were used, all in apparently normal condition.

Each experiment consisted of the following procedure: (1) The body weights of 6 normal animals were obtained. (2) Initial heart rates and estimates of the degree of contraction were obtained, using a method of illumination already described.[6] (3) An estimate of the amount of crop peristalsis was noted. (4) Meanwhile, solutions or emulsions, stabilized with 10 mg. Witte's peptone per 5 cc. emulsion, were prepared in 1, 2, 4, 6, 8 and 10% concentrations in Hob-

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[1] Richardson, C. H., and Smith, C. R., *U. S. D. A. Bull.*, 1160, 1923.

[2] Moore, Wm., *J. Agr. Res.*, 1917, **9**, 371.

[3] Holt, Jos. J. H., *Lancet*, 1916, **1**, 1136.

[4] Campbell, F. L., *J. Gen. Physiol.*, 1926, **9**, 433.

[5] Hockenyos, G., *J. Ec. Ent.*, 1932, **25**, 253.

[6] Yeager, J. Franklin, *Ann. Ent. Soc. Am.*, 1931, **24**, 739.

son's solution[7]; the latter consists of 0.161 M NaCl, 0.003 M KCl and 0.002 M $CaCl_2$. (5) The animals were injected through the conjunctiva of the coxa-femur joint of the left third thoracic leg by means of an improvised, capillary injection pipette (glass) of 0.1 cc. capacity and graduated to 0.001 cc. Each animal was injected with a single concentration in amount equal to 10% of its body weight. (6) The animal was immediately observed for (a) general activity, (b) rate of locomotion, (c) maintenance and recovery of equilibrium, (d) activity of antennae and mouth-parts, (e) activity of legs (excepting the injected leg), (f) body twitches, (g) rate and degree of heart contraction, (h) activity of crop and (i) body muscle twitches (thorax). Observations (g), (h) and (i) were made with transmitted light. (7) Each animal was thus injected and observed. (8) Later the same observations were again made upon each animal, so that all animals were subjected to 2 complete post-injection examinations.

The compounds used were benzene, furan; toluene, sylvan; orthoxylene, 2.5-dimethylfuran; nitrobenzene, 2-nitrofuran; furfuryl alcohol; benzyl alcohol; furfural, benzaldehyde; ethyl benzoate; ethyl furoate; sodium benzoate, sodium furoate; acetophenone, furyl methyl ketone; benzoyl chloride and furoyl chloride. These compounds were all in chemically pure form.*

The results have been summarized in Table I. Except for experiments 6, 9, 16, and 18, the corresponding concentrations are arranged into 2 groups. Control experiments indicated that the injection of Hobson's solution up to 15% body weight or of the saline plus an amount of peptone equivalent to that used in the experiments is without injury to the animal.

Analysis of these results has indicated no consistent relationship between chemical constitution and physiological effect. In general, however, it would seem that those compounds, in the relatively low concentrations used, permitting general activity to continue in an apparently "purposeful" manner fall into 2 groups: (1) compounds with the methyl radical substituted into the ring (toluene, sylvan, dimethyl furan, orthoxylene) and (2) the acid derivatives or esters and acid chlorides (furfuryl alcohol, Na benzoate, ethyl benzoate, Na furoate, ethyl furoate, benzyl chloride). The methyl substituted compounds would appear to be relatively less toxic and to permit relatively earlier recovery.

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[7] Hobson, A. D., *Br. J. Exp. Biol.*, 1928, **5**, 385.

* The authors are indebted to Dr. Henry Gilman of Iowa State College for the furan derivatives used in these experiments.

TABLE I.

Exp. No.	Substance	Concn. in Hobson's soln.	Activity	Type Behavior	Equilibrium	Locomotion	Legs			Body twitches	Body muscle twitches	Heart		Crop
							1st pair	2nd pair	3rd right leg only			Rate	Degree contracting	
1	Benzene	1-4	—	X	—	—	N	N	N	+		+	—	—
		6-10	—	X	—	—	N	N	—	+	+	—	—	—
4	Furan	1-4	—	X	—	—	N	—	—	+	+	—	—	+
		6-10	—	X	—	—	N	—	—	+	+	—	—	—
2	Toluene	1-4	N	P	+	—	N	N	N	—	—	+	N	
		6-10	N.	P	+	N	N	N	N	—	—	+	N	N
3	Sylvan	1-4	S	P	+	S	N	N	N	—	—	—	—	+
		6-10	—	X	—	—	N	N	—	+	+	—	—	—
6	Orthoxylene	1-8	—	P	+	—	N	N	N		+	—	—	N
		10	—	X	—	—	—	—	—		—	—	—	—
5	2.5-Dimethyl Furan	1-4	+	P	+	—	N	N	N	—	—	N	—	+
		6-10	—	X	—	—	N	—	—	+	+	—	—	—
7	Nitro-benzene	1-4	—	X	—	—	N	—	—	+	+	—	—	—
		6-10	—	X	—	—	N	—	—	+	+	—	—	+
8	Nitro-furan	1-4	—	X	—	—	—	—	—	—	—	—	—	—
		6-10	—	X	—	—	—	—	—	—	—	—	—	—
10	Benzyl Alcohol	1-4	—	X	—	—	—	—	—	—	—	—	—	—
		6-10	—	X	—	—	—	—	—	—	—	—	—	—
9	Furfural Alcohol	1	N	P	+	—	N	—	—	+	+	—	—	N
		2-10	—	X	—	—	—	—	—	—	—	—	—	—
11	Benzaldehyde	1-4	—	X	—	—	—	—	—	—	—	—	—	—
		6-10	—	X	—	—	—	—	—	—	—	—	—	—
12	Furfural	1-4	—		—	—	—	—	—	—	—	—	—	
		6-10	—		—	—	—	—	—	—	—	—	—	
14	Ethyl benzoate	1-4	—	P	—	—	N	N	—	—	—	+	—	—
		6-10	—	X	—	—	—	—	—	+	+	—	—	—
16	Ethyl furoate	1-6	—	P	—	—	N	—	—	—	—	+	—	—
		8-10	—	X	—	—	—	—	—	—	—	—	—	—
13	Sodium benzoate	1-4	N	P	+	N	N	N	N	—	—	+	—	—
		6-10	—	X	—	—	—	—	—	—	—	—	—	—
15	Sodium furoate	1-4	—	P	—	—	N	N	—	—	—	+	+	+
		6-10	—	X	—	—	—	—	—	—	—	+	N	—
17	Acetophenone	1-4	—	X	—	—	—	—	—	—	—	—	—	—
		6-10	—	X	—	—	—	—	—	—	—	—	—	—
20	Furyl Methyl Ketone	1-4	—	X	—	—	—	—	—	+	+	+	—	—
		6-10	—	X	—	—	—	—	—	—	—	—	—	—
18	Benzyl Chloride	1	N	P	+	N	N	N	N	—	—	+	—	—
		2-10	—	X	—	—	—	—	—	—	—	—	—	—
19	Furoyl Chloride	1-4	—	X	+	N	—	—	—	—	—	—	—	—
		6-10	—	X	+	—	—	—	—	—	—	—	—	—

Physiological effects of injecting into the cockroach, *P. orientalis*, quantities equal to 10% of body weight of relatively low (1, 2 and 4%) and relatively high (6, 8 and 10%) concentrations of various benzene and furan derivatives dissolved in or emulsified in Hobson's solution (0.161 M NaCl, 0.003 M KCl, 0.002 M $CaCl_2$) using 10 mg. Witte's peptone per 5 cc. emulsion as a stabilizing agent.

The symbols used in the table have the following meanings:

N = unchanged or normal, *i. e.*, negligible effect.

P = purposeful behavior

X = purposeless behavior.

+ = increase in activity, recovery of equilibrium, occurrence of twitches, increase of heart rate or degree of contraction.

— = opposite effect of +.

S = occurrence of spasms.

Several points of physiological interest were noted during the experimental work that are not shown in the table. (1) In most animals a decrease in activity was accompanied by an obvious "purposeless" type of behavior, but in some animals a decrease in activity was associated with a "purposeful" behavior (Na benzoate, ethyl benzoate, Na furoate, ethyl furoate, benzyl chloride, furfuryl alcohol). The activity was observed to be in no case normal or increased and at the same time purposeless. (2) Immediately following the injection of sylvan, dimethyl furan (4 and 6%), orthoxylene or furfuryl alcohol, the animal became quiescent or completely inactive; following this short period of quiescence, activity was again resumed. (3) In experiments where observation of the animals could be extended, recovery was noted to occur in the cases of benzene, toluene, furan, sylvan, nitrobenzene (incomplete recovery), benzyl alcohol (1% only) and benzaldehyde (1% only). (4) The opacity of the animal to transmitted light was increased by benzene, benzaldehyde, nitrobenzene, and furfural. In some cases, the increase of opacity was sufficient to interfere seriously with observations. (5) The orderly contractions of the heart were changed to a disorganized functioning by benzene, sylvan, nitrobenzene, nitrofuran, furfuryl alcohol, benzyl alcohol, benzaldehyde, furfural, Na benzoate, ethyl benzoate, furoyl chloride, furyl methyl ketone; and by Na furoate (2%), ethyl furoate (8%) and benzyl chloride (2 and 4%). The disorganization of heart function occurred in several different ways: (a) thoracic and abdominal portions contracted at different rates and to different extents; (b) contractions of extreme anterior end of heart only or of extreme posterior end only or of both extremes only; (c) reversal of heart beat in the case of furyl methyl ketone. (6) In a number of animals, in which injection was followed by complete cessation of heart beat, recovery of heart beat appeared first at the posterior end of the heart and then gradually progressed anteriorly.

Although injection into the body cavity may be expected to result in effective contact between injected fluid and the various body tissues, due to the presence of an open circulatory system, the reactions of the animals seem to indicate that the primary effect is upon the nervous mechanism.

Summary. Various benzene and furan derivatives, dissolved or emulsified in Hobson's solution, have been injected quantitatively into the cockroach, *Periplaneta orientalis* (Linn.). The effects of the injections upon various body activities are given in condensed and tabular form. No clear-cut relationship between chemical structure

and physiological effect has been detected, although the methyl groups appear to be associated with lesser toxic effects. Several observations of physiological interest are described.

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Estimation of Ethanol in the Embalmed Brain.

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Methods for the quantitative determination of ethanol in the brain have required that the tissue be fresh. A method has been devised in the hope that ethanol may be estimated after embalming of the body. The procedure of Gettler and Tiber,[1] is modified as follows: The tissue is steam distilled. The distillate is slowly refluxed with H_2SO_4 and $K_2Cr_2O_7$ for 30 minutes, prior to distillation. This completely oxidizes the formaldehyde and methanol to CO_2 and H_2O, but the oxidation of ethanol is carried only to acetic acid. After distillation the acetic acid is determined by titration against N/20 NaOH. The ethanol in the tissue is calculated by the formula: 1 cc. of N/20 NaOH = 2.3 mg. ethanol. By this procedure ethanol can be accurately determined in a standard mixture of ethanol, methanol, formaldehyde and water.

Following the preliminary chemical study, the investigation was extended to 4 series of rabbits. Ten rabbits were given 6 gm. ethanol per kilo weight, by stomach tube and were killed 5 hours later by the neck stroke. The ethanol content of the brain varied from 0.36% to 0.45% with an average of 0.40%. A second group of 10 rabbits received the same treatment but were embalmed before analysis of the brains for ethanol. The figures varied from 0.21% to 0.52%, with an average of 0.34%.

A third group of 5 rabbits were killed and embalmed without the administration of ethanol. The figures for this series were practically identical with the figures from a fourth series of 5 control rabbits which were killed and whose brains were analyzed immediately for ethanol, namely about 0.005%.

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[1] Gettler, A. O., and Tiber, A., *Arch. Path. and Lab. Med.*, 1927, **3**, 75.

6342

Glycogen Formation in Rats After Administration of Diets High in Galactose, Glucose, and Lactose.

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It has been shown[1] that galactose exerts a greater ketolytic action than does glucose when administered to fasting humans or to subjects on a protein-fat diet. Moreover, a superior nitrogen sparing action was usually noted after the ingestion of galactose than that found after a similar amount of glucose. In both cases the better effect of galactose was demonstrated not only by the lower level to which these constituents were depressed but also by the more prolonged persistence of the effect. One explanation for this discrepancy is that the glycogen content of the animal may remain at a higher level for a longer time after galactose than after glucose administration. Cori,[2] however, found that galactose was a very poor glycogen former in rats as compared with glucose although he made no studies at longer than 4-hour intervals after the sugars were fed.

This investigation was made to ascertain the quantitative relationships in glycogen storage when longer periods of starvation had elapsed following the administration of diets high in glucose, galactose, lactose, or a mixture of glucose and galactose. Besides the sugar under investigation which comprised 44 to 46% of the diet, casein (20%), yeast (10%), lard (20%), salt (4%), and cellu (2%) made up the food. Adult male rats were used. Three rats of each group of 6 served as controls, while the remaining ones were fed galactose after an appropriate period of fasting following the administration of the above diet. The galactose was fed by stomach tube in 50% solution in a dose of 0.50 gm. per 100 sq. cm. of body surface. The control animals were killed at the same time as the galactose fed ones. Amytal was used as an anesthetic. The tissues were immediately frozen in a CO_2 snow-ether mixture after being removed from the body and weighed in this condition. Glycogen was determined by a combination of the Pfluger method and the Shaffer-Hartmann procedure.

The glycogen content of both liver and muscles of the galactose-

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[1] Deuel, H. J., Jr., Gulick, M., and Butts, J. S., *J. Biol. Chem.*, 1931, **92**, 23.

[2] Cori, C. F., *J. Biol. Chem.*, 1926, **70**, 577.

TABLE I.
Glycogen content of 3 fasted rats.

Previous diet	Food intake per rat gm. daily	Liver %	Muscle %	Heart %
33 hr. fast				
Glucose	10.2	0.13	0.19	0.59
Galactose	14.1	0.37	0.29	0.52
Galactose-Glucose	18.7	2.12	0.32	0.46
54 hr. fast				
Glucose	19.1	0.23	0.19	
Galactose	17.5	0.77	0.32	
Lactose	8.2	0.39	0.28	

TABLE II.
Glycogen content of 3 fasted rats after galactose administration.

Previous diet	Food intake per rat gm. daily	Liver %	Muscle %	Heart %	Galactose retained %
24 hr. fast before galactose. Animals killed 9 hrs. later					
Glucose	12.6	1.53	0.27	0.64	52.6
Galactose	10.9	2.23	0.28	0.70	52.1
Glucose-Galactose	9.9	1.80	0.27	0.68	48.3
48 hr. fast before galactose. Animals killed 6 hrs. later.					
Glucose	17.4	1.26	0.28		60.8
Galactose	16.2	1.56	0.34		63.2
Lactose	8.1	0.97	0.29		68.1

fed control animals far exceeded that of the glucose control rats, although no distinctive differences were found in the case of heart glycogen. In addition the rats which received galactose after a previous fasting period had higher values for liver glycogen if they had previously been on a galactose diet than if they had eaten the glucose diet. The muscle glycogen in these animals exceeded that of the fasting glucose controls but approximated the value of the fasting galactose control animals. The results with lactose are intermediate. One very high result was found with the galactose-glucose series which may be accounted for by the high food intake of these animals. The results after the administration of galactose make it evident that considerable glycogen may be formed after galactose is given. The higher glycogen values for the galactose-fed animals can not be attributed to higher food intake of these rats. In only one case of the 4 groups of animals was the intake of food higher in the galactose rats than in the glucose animals. This does not take into consideration, moreover, the loss of galactose in the urine. Further experimental work is being made at shorter and longer intervals of fasting.

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Hypophysis and Detoxification.

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Previous work has shown that the removal of the anterior lobe of the hypophysis not only prevents further growth of the normal tissues of the animal, but diminishes the rate of growth of tumors.[1] Yet in spite of the small size of the tumors the animals very often do not live any longer than the controls. Animals with the hypophysis removed also succumb to pneumonia and other infections more frequently than normal.

Providing the anatomical changes are associated with similar changes in function, this result would be expected from the work of Smith[2] demonstrating that pituitary removal causes atrophy of the adrenal cortex. Lewis[3] first showed that adrenalectomy lowered the resistance of the animals to toxins. Recently Perla and Marmorston-Gottesman[4] have shown that extracts of the adrenal cortex which preserve life in adrenalectomized animals also increase the bodily resistance to toxins. On the other hand, Cutler[5] reported that removal of the hypophysis had no effect on the immune reaction of guinea pigs to *bacillus typhosis*. However, with his method of operation no animals survived which had complete operations. The immunity studies were therefore carried out with partially hypophysectomized animals. The following experiments were undertaken to determine the detoxifying ability of completely hypophysectomized rats as compared with similar normal animals.

The toxin used was a solution of crystalline cobra venom. Doses of this toxin can be accurately duplicated over a long period of time, a condition not true for the bacterial toxins. The crystalline cobra venom was dissolved in normal physiological saline solution to make a concentration of 100 mg. of venom per litre. This solution was injected intraperitoneally into albino rats raised in our own colony. Preliminary experiments with normal animals established the minimum lethal dose at slightly more than 0.075 mg. per 100 gm. body weight. The ratio of minimum lethal dose to body weight was constant over a wide range.

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[1] Ball, Howard A., and Samuels, L. T., *Am. J. Cancer*, 1932, **16**, 351.

[2] Smith, P. E., *J. Am. Med. Assn.*, 1927, **88**, 158.

[3] Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 506.

[4] Perla, D., and Gottesman, J. M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 474.

[5] Cutler, E. C., *J. Exp. Med.*, 1922, **35**, 243.

The hypophysectomized rats were prepared by the method of P. E. Smith, the sphenoid being trephined through the nasopharynx. The entire gland was aspirated without touching the brain. All of the animals had been operated at least 3 weeks before the tests and were definitely free from any post-operative complications. Thirteen operated animals were used: 8 of these received one-sixth grain of thyroid twice a week for 2 weeks previous to injection of the toxin to maintain the basal metabolic rate and eliminate the thyroid changes as a possible factor. Operated rats given doses of 0.043 mg. per 100 gm. body weight all survived, but when the total injection was 0.05 mg. per 100 gm. body weight, 10 animals died and 3 survived. Upon autopsy all of the animals which died were found to have complete operations. One of the surviving animals had portions of the anterior lobe still present. The minimum lethal dose for hypophysectomized animals was 0.05 mg. per 100 gm. body weight.

The detoxifying ability of hypophysectomized rats is therefore only ⅔ that of similar normal animals. The difference is even greater than the figures would indicate since the hypophysectomized animals had much smaller amounts of body fat. This effect of removal of the hypophysis is not due to the indirect effect of a lowered thyroid function but may be due to a reduced function of the adrenals.

6344

Sexual Variation in the Carbohydrate Metabolism of Rats.

H. J. DEUEL, JR., L. SAMUELS AND M. GULICK.

From the Department of Biochemistry, University of Southern California School of Medicine, and the College of Medical Evangelists, Los Angeles.

A distinct variation exists in the rate of development and the extent to which ketosis occurs in normal men and women during the early days of fasting.[1] The present investigation was undertaken to determine whether such a difference can be ascribed to a more rapid depletion of the carbohydrate supply of the female.

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[1] Deuel, H. J., Jr., and Gulick, M., *J. Biol. Chem.*, 1932, **96**, 25.

Rats were used as experimental animals. In order to insure a uniform content of carbohydrate at the beginning of the test, the animals were first fasted for 48 hours after having been previously fed on an adequate normal diet. Glucose was then administered by stomach tube in 50% solution, 0.500 gm. per 100 sq. cm. of body surface. The animals were again fasted for 24, 36, 48 or 72 hours, then anesthetized with amytal and the whole liver, a sample of muscle and the heart were quickly removed and frozen in a mixture of CO_2 snow and ether. Glycogen was determined by a combination of the Pfluger method and the Shaffer-Hartmann procedure.

The glycogen content of the livers of mature female rats was invariably lower than that of mature males. However, no significant difference in the glycogen store in the liver, muscle or heart of sexually immature male and female rats was found. In castrated female animals as high or higher values were found as in males. In a series of 50 castrated female rats, half of which received sufficient theelin several times daily over a period of a week to keep them continuously in active oestrus, it was found that the injected animals behaved as normal females in respect to their glycogen stores. It is concluded that a sexual difference exists in the rate at which the carbohydrate stores are depleted in fasting. This variation is not noted before sexual maturity or after castration of the female. It is restored, however, in female castrates by the injection of theelin.

6345

A Roentgen-Ray Study in Absorption of Thorium Dioxide from Peritoneal Cavity of Albino Rat.*

LEON J. MENVILLE AND J. N. ANÉ.

From the Radiological Division, Department of Medicine, Tulane University.

It has long been known that serous cavities have the capacity of absorbing certain injected substances. Cunningham[1] showed that, when a mixture of erythrocytes, large unfiltered carmine granules, and fine lampblack granules, made up in isotonic NaCl solution, was injected into the peritoneal cavity of animals, all 3 types of material reached the anterior mediastinal glands shortly after injection. His results indicate that most, if not all, of the transfer of granular material from the peritoneal cavity into the diaphragmatic lymphatics, during the first 30 minutes, takes place by means of a type of phagocytosis. His important findings give a clearer understanding of the high phagocytic capability of the lymphatic endothelial cells to remove large amounts of particulate matter from the peritoneal cavity. The diaphragm is rich in lymphatics and its lymphatic vessels anastomose with the lymphatic vessels of the

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* Aided by a grant from the David Trautman-Schwartz Research Fund.

[1] Cunningham, R. S., *Am. J. Physiol.*, 1922, **62**, 248.

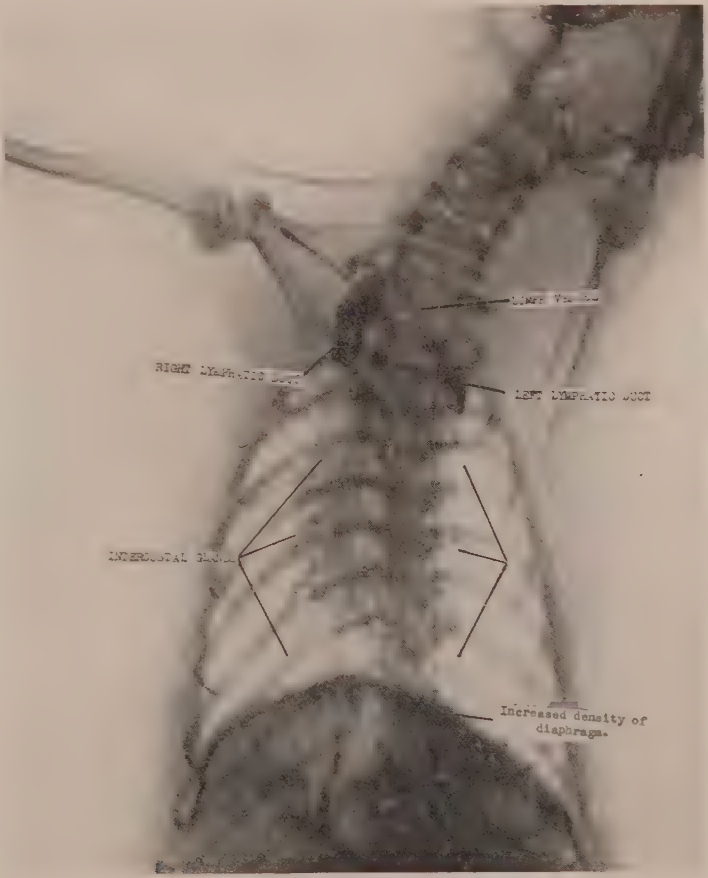

FIG. 1.
Enlargement from original film showing the connection between the lymphatic system of the diaphragm and the lymphatic ducts by way of the intercostal lymph nodes and vessels. The two lymphatic ducts are apparently connected by a lymph vessel.

pleura and the peritoneum, which, in turn, connects with the right and left lymphatic ducts. The thoracic duct, terminating in the internal jugular vein, conveys the great mass of lymph and chyle into the blood. It is the common trunk of all lymphatic vessels below the diaphragm. The right lymphatic duct receives lymph from the right upper extremity, the right thorax, right lung, right side of the heart, and from parts of the convex surface of the liver. It opens into the junction of the internal jugular and the subclavian veins separately. Absorption in this region is extremely rapid due to this network of lymphatics. The practical application of this knowledge is found in the Fowler position, whereby, upon elevating the head of the bed after operation for abdominal infection, the infected material is permitted to gravitate toward the pelvic peritoneum, which absorbs with comparative slowness, while the peritoneum in the upper abdomen absorbs very rapidly.

Our studies on the absorption of thorotrast, a colloidal thorium dioxide, from the peritoneal cavity of albino rats were undertaken to visualize, if possible in the living, by means of the roentgen ray, the routes of absorption by the lymphatic system from the peritoneal cavity to the right and left lymphatic ducts.

Three healthy albino rats were injected intraperitoneally with 0.5 cc. thorotrast. Roentgenograms made 24 hours after injection and again 2 weeks later showed very fine striations in the abdomen which closely resembled lymph vessels, previously reported by us.[2] The diaphragm showed an increased density, suggesting some absorption of the thorium in this region, probably by the lymphatics. Chest films showed clearly the intercostal glands and vessels and also the connection of these lymph vessels with the right and left lymphatic ducts, also the drainage system of the lymphatics from the peritoneal cavity, diaphragm, intercostal nodes and vessels, into the lymphatic ducts. In one, an apparent connection was observed between the 2 lymphatic ducts (Fig. 1). Whether this is but a branch of a duct, we do not know. The intraperitoneal injection of thorotrast did not injure the rats.

We believe this to be the first time that this portion of the lymphatic system has been visualized with the roentgen ray.

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[2] *J. Am. Med. Assn.*, May 21, 1932, **98**, 21, 1796.

6346

Differentiation by Cataphoretic Velocity of Fresh and Old Laboratory Strains of H. Pertussis.*

G. S. SHIBLEY. (With the technical assistance of Helena Hoelscher.)

From the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland, Ohio.

In connection with studies concerned with the etiology of whooping cough we have examined the serological character of *H. pertussis.* Krumwiede, Mishulow, and Oldenbusch[1] have described 2 fixed serological varieties which they have designated as types A and B. Very recently Leslie and Gardner[2] have restudied the problem and have concluded that *H. pertussis* is a uniform species without fixed varieties or types when first isolated from the human subject, but that after subculture upon various laboratory media, it passes through a series of antigenically distinct phases which they have called Phases I, II, III, and IV. They believe the first 2 phases correspond to the smooth, and the last 2 to the rough varieties of other bacteria.

We have isolated *H. Pertussis* from early typical cases of whooping cough by the cough plate method and have studied 16 such fresh strains. In addition we have made observations on various old stock strains kindly provided us by Dr. Park in New York and Dr. Wadsworth in Albany. We have confirmed the observations of Leslie and Gardner to the extent that we have been able by agglutinative, absorptive, and toxicity studies to demonstrate the uniformity of all fresh strains obtained by us in Cleveland and to show that they pass into antigenically different phases upon continued subculture. The determination of the toxicity of strains has been made upon rabbits by the skin test method using filtrates of the organism rather than by the use of guinea pigs as carried out by Leslie and Gardner. We have not been able to divide the later phases into the 3 sharp categories described by these authors. We are still working upon this aspect of the problem.

The chief object of this communication is the reporting of the fact that freshly isolated "Phase I" strains may be rapidly and accu-

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* Aided by a grant from the Rockefeller Foundation.

[1] Krumwiede, C., Mishulow, L., and Oldenbusch, C., *J. Infect. Dis.*, 1923, **32**, 22.

[2] Leslie, P. H., and Gardner, A. D., *J. Hygiene,* 1931, **31**, 423.

rately distinguished from all our old laboratory phases ("Phases II, III, and IV") by means of their cataphoretic migration velocities. Cataphoretic migration velocities were determined by means of the Northrop-Kunitz[3] micro-cataphoretic apparatus and are recorded in terms of microns, per sec., per volt, per cm. Before making readings the bacteria were washed three times in distilled water and then were suspended in glycocoll-sodium phosphate-sodium acetate buffer solution[4] at pH 7.0, 1/100 molar concentration.

All new strains have shown much slower migration velocities than the old laboratory strains and when these newly isolated strains ("Phase I") have passed over to the later phases, they have shown the much more rapid migration velocities that have characterized our old laboratory strains. This change in velocity has regularly followed the acquisition on the part of these new strains of the ability to grow upon heated blood agar. The migration velocities of all freshly isolated strains, read under standard conditions, have ranged between 1.2 and 2.1μ/sec./V/cm., average 1.6. The migration velocities of all old strains studied have varied between 3.3 and 4.6μ/sec./V/cm., average 3.9. Readings made upon our fresh strains which have passed over to the "laboratory phases" have averaged over 4.0μ/sec./V/cm.

Difference between the cataphoretic velocities of virulent and avirulent ("R" and "S") strains of various bacteria has already been noted. The differences here reported between the migration rates of freshly isolated and of old laboratory strains would seem to suggest the probability that the latter may have changed sufficiently antigenically as to be classified as rough or avirulent forms. So far all efforts to show morphological "R" and "S" colonies on many varieties of media have failed. It is interesting to note that cross "Schwartzman Phenomenon" tests have failed to indicate antigenic differences between freshly isolated and old laboratory strains.

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[3] Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1925, **7**, 729.

[4] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921, **4**, 639.

6347

Relation of Humidity to Resistance to Infection in Rachitic Rats.

ELIZABETH CHANT ROBERTSON AND C. B. WELD. (Introduced by F. F. Tisdall.) (With the technical assistance of M. Elizabeth Doyle.)

*From the Research Laboratories, Sub-Department of Paediatrics, University of Toronto, and the Hospital for Sick Children, Toronto.**

McDowell,[1] showed that the mortality following an intraperitoneal injection of pneumococci in adult rats kept for 2 weeks in a room at a temperature of 84°F., was much higher when the relative humidity was 84% than when it was 44%. When the temperature varied between 65° and 72°F., a larger percentage of all the rats died, but variations in the humidity had no effect. Kligler and Olitzki[2] reported very similar results with adult mice, but, in their work, the exposure to the special environment took place only 3 days before and for one month after an oral infection with *Salmonella enteritidis.* At 68°F. differences in humidity did not influence the mortality rate.

The following experiments differ from those above mentioned in that (1) young rats were used; (2) a rickets-producing diet was fed; (3) the exposure to the various temperatures and humidities was for the 4 weeks preceding infection; (4) the effect of air drawn from inside and outside the building was compared. Litters of rats which had just been weaned were divided into 4 equal groups, and put in divided cages in 4 closed wooden boxes through which the conditioned air was passed. The air for 2 of the boxes, one high and one low humidity, was drawn from outside the building, and that for the other 2, also one high and one low humidity, was drawn from the large animal room which housed the boxes. The air for each pair of boxes was drawn from its source by a pump and passed through a tank of anhydrous crude calcium chloride, through an electric heater, and into the box. The heaters were under the control of thermo-regulators in the boxes, and the temperature of the conditioned air surrounding the rats was kept constant to within a few degrees. This warm dry air was flowing at the rate of about 30 liters per minute to each box, enough to ensure a complete change of air in each box every 10 minutes. A small jet of steam was led into the intake pipe of one box of each pair, and it was found that this almost saturated the atmosphere in those boxes with water vapor without appreciably raising the temperature. Except for about one hour of the 24 daily, when the boxes were open for feeding, cleaning or inspection, the air conditions were kept fairly constant. The low humidity boxes were set at 83°-84°F. and the air entering them was 21% saturated with water vapor in the case of the one receiving outside air, and 24% saturated in the one receiving inside air. The high humidity boxes were somewhat less constant but maintained temperatures of about 81° F. and humidities of 80% to 100%, usually the latter. The rats were fed a modification of Steenbock's[3] rickets-producing diet, which contained considerable amounts of vitamins A, B and E, as follows: dried ordinary bread, 12½%; yellow corn, 59½%; wheat gluten, 17%; wheat germ, 6%; calcium carbonate, 3%; sodium chloride, 1%; machine dried alfalfa, 1%. The animals showed a marked degree of rickets after 28 days on this diet. The rate of growth was affected very slightly, if at all, by the high temperature, and the rats in all 4 boxes grew equally well.

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* Under the direction of Alan Brown.

[1] McDowell, C., *Am. J. Hyg.*, 1923, **3**, 521.

[2] Kligler, I. J., and Olitzki, L., *Am. J. Hyg.*, 1931, **13**, 349.

After the rats had been kept under these conditions for 28 days, they were starved for 7 hours, put in individual cages, and fed a small amount, 0.0029 cc. (0.1 cc. of a 1 in 35 broth dilution) of an 18 hours' culture of *Salmonella muriotitis*,[4] a strain of *Salmonella enteritidis*, on a cube of dried bread. After infection the rats were kept at room temperature in individual cages for 28 days, when the experiments were terminated, and the degree of resistance was estimated by the percentage of each group which survived. All of the dead rats except one, which was in a state of decomposition, yielded *Salmonella muriotitis* from the heart's blood, usually in pure culture. The organism was identified by its non-utilization of lactose, and by a positive slide agglutination in a dilution of 1 in 100. No gross pathology other than that usually seen in this infection[5] was found. The livers and spleens of the survivors were cultured, but only one liver culture yielded *Salmonella muriotitis*.

TABLE I.

	Relative Humidity					
	21%–24%			80%–100%		
	No. Rats	No. Survived	% Survived	No. Rats	No. Survived	% Survived
"Inside" Air	9	5	55	10	0	0
"Outside" Air (April 18-May 17)	9	6	66	8	2	25

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[3] Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

[4] McCordock, H. A., *Bull. Johns Hop. Hosp.*, 1925, **37**, 412.

[5] Robertson, E. C., and Ross, J. R., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 999.

From the table it is seen that a much larger percentage of the rats kept at the lower humidity survived. Also, the rats which were supplied with air from outside the building withstood the infection slightly better, especially when the humidity was high, but whether this difference is of real significance will be further investigated.

In a similar preliminary experiment, the details of which are not given, too small a dose of the bacterial culture was fed, with the result that the organism was recovered from the cardiac blood of only one-third of the dead rats, and the mortality throughout was lower, but the deleterious effect of the high humidity was again evident, as 74% of 18 rats kept in the dry air, and 52% of 21 rats kept in the moist air survived.

Conclusions. When kept at a high external temperature (83°F.), young rachitic rats exposed to a relative humidity of 22% for 4 weeks survive a subsequent oral enteritidis infection in much larger numbers than litter mates exposed to a relative humidity of 90%.

These findings are similar to those reported by McDowell and Kligler.

6348

I. Penetration Through Tissue of Iodine in Different Solvents.*

M. S. BISKIND. (Introduced by W. F. Von Oettingen.)

From the Department of Pharmacology, Western Reserve University.

It has been shown by Karns[1] and by Karns, Cretcher and Beal,[2] that certain aqueous solvents for antiseptic iodine preparations are superior to those commonly in use, with respect to the iodine adsorption behavior at surfaces. This controls surface dosage, and hence becomes a very important factor in germicidal effectiveness[3] and in penetration through tissues. It has been further demonstrated by Karns[4] that the quantity of iodine absorbed on a treated surface is not a necessary function of the concentration of the iodine in the solvent used, and that surface tension plays no important rôle in this reaction. Changes in the iodine-iodide ratio of the aqueous solutions were found considerably to affect the iodine deposition.

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* Contribution from the Mellon Institute Fellowship for Research in the Pharmacology of Iodine.

[1] Karns, G. M., *J. Am. Pharm. Assn.*, 1932, **21**, 779.

[2] Karns, G. M., Cretcher, L. H., and Beal, G. D., *J. Am. Pharm. Assn.*, 1932, **21**, 783.

[3] Gershenfeld and Miller, *J. Am. Pharm. Assn.*, 1932, **21**, 894.

[4] Karns, G. M., *loc. cit.*

In the work presented here, a series of iodine solutions of different concentrations and in various solvents were compared under standard conditions with respect to their rates of penetration through frog skin.

Only the fresh non-pigmented skin from the ventral body surface of large frogs between the pectoral and pelvic girdles was used, in segments about 2 cm. square, as this was found to be more nearly uniform in texture and thickness than skin from any other part of the body. It was found that any attempt to tie the skin onto containers resulted in areas of increased permeability at the sites of greatest tension, thus materially modifying the results. However, merely laying the skin, outside in, over the end of a half-inch glass tube sufficed, in the majority of cases, to seal it when inverted. Stretching was carefully avoided. The end of the tube closed with the skin was dipped in starch-iodide solution containing 0.65% of sodium chloride, a drop of the latter remaining suspended therefrom. Just enough iodine solution was placed in the tube to cover the skin and the time required for the first appearance of a blue color in the starch solution was recorded. If the stain on the skin was later found to have extended beyond the inside margin of the tube, that observation was discarded.

To minimize errors due to differences in various skins, segments of the same skin were at first used with 2 or 3 different solutions simultaneously. It was found, however, that the range of variation with different skins was no greater than that on the same skin, so that later observations were made without regard to this factor.

The solutions tested and the results obtained are given in the tabulation. The 1.85% aqueous iodine solutions were supplied by

TABLE I.

	Solutions	Mean Time for Penetration in Seconds/100†	No. Observations
1.	I_2 5.0%, NaI 10.0%, in water	0.9±0.05	15
2.	1.85%, NaI 2.20%, in water	2.1 0.15	15
3.	1.85%, KI 2.42%, in water	2.7 0.039	15
	1.85%, NaI 2.05%, KI 0.089%, CaI.6H_2O 0.081%, in water	2.8 0.28	15
4.	3.5%, KI 2.5%, in 92.5% alcohol	3.6 0.19	15
5.	7.00%, KI 5.0% in 90% alcohol (U.S.P. Tinc.)	4.5 0.094	15
6.	1.85%, KI 1.33%, in 93.3% alcohol	11.3 0.52	9

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† In this, as in the subsequent report, I am greatly indebted to Dr. Norman Reider for assistance with the statistical analysis of the data.

Dr. G. M. Karns. These are approximately isotonic for mammalian and hence somewhat hypertonic for frog tissue.

It will be seen that the aqueous solutions are all superior in speed of penetration to the tinctures. This holds true even in comparing the 1.85% aqueous solutions with the U. S. P. Tincture containing 3.8 times as much iodine. When the U. S. P. Tincture is diluted with 95% alcohol to an equal iodine content, the tremendous rôle played by the solvent immediately becomes evident. The greater iodine content and the marked hypertonicity of the 5% aqueous solution are undoubtedly involved in its greater speed of penetration as compared with the other aqueous solutions.

6349

II. Effect of Iodine in Different Solvents on Permeability of Frog Skin to Ringer's Solution.*

M. S. BISKIND. (Introduced by W. F. Von Oettingen.)

From the Department of Pharmacology, Western Reserve University.

In the previous report,[1] a series of iodine solutions were compared with respect to their rate of penetration through frog skin. In this study an attempt was made to compare some of these solutions, and others, as to their relative effects on the ultrafiltration permeability of frog skin to Ringer's solution, with a view to obtaining some information as to the tanning properties of these solutions.

The method used is a modification of that described by Sollmann[2]: The skin was stripped off the hind limbs of large frogs. separated, tied off at the ankles, reinverted and tied onto the lower ends of filtration tubes, each consisting of a stoppered burette connected by means of a T-tube containing a Mariotte stopper, with a glass tube, one-half inch in diameter and of sufficient length that filtration could be carried out for several hours under a constant pressure of 31 cm. of water. The outside of the skin was exposed to the air.

The outside surface of the skin was treated by immersion in one of the various solutions for exactly 5 minutes after which it was washed off with tap water. The tube was then filled with Ringer's solution, stoppered, and the amount passing through the skin for a 3-hour period determined. The latter interval was adopted as this is nearly the maximum time during which filtration proceeds at a nearly constant rate. Beyond this period there is usually an increase in rate, indicative, presumably, of a marked decrease in the viability of the cells. Shorter periods involve a considerable error in estimating the small quantities of fluid which pass the skin. Usually a treated and untreated leg from the same frog were run parallel at the same time, but inasmuch as the results with the treated skins were quite uniform, the controls are all grouped together in the tabulation.

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* Contribution from the Mellon Institute Fellowship for Research in the Pharmacology of Iodine.

[1] Biskind, M. S., this journal

[2] Sollmann, T., *J. Pharmacol. Exp. Therap.*, 1922, **21**, 200.

The solutions used and the results are as follows:

Solutions	Filtration cc. per 3 hours	No. Observations
1. 7.0% U.S.P. Tincture Iodine	1.5±0.1	8
2. I_2 1.85%, NaI 2.20%, in water	2.1 0.026	7
I_2 1.85%, NaI 2.05%, KI 0.089% CaI.6H_2O 0.081%, in water	2.1 0.16	7
I_2 5.0%, NaI 10.0%, in water	2.2 0.31	7
I_2 1.85%, KI 2.42%, in water	2.2 0.34	6
3. Normal (untreated)	11.25 0.55	70
Mercurochrome 2% aqueous	11.5 1.43	7
4. Ethyl alcohol 95%	18.2 1.12	8

The aqueous solutions all closely approach the 7% U. S. P. Tincture in reducing the permeability of these skins. There is no notable difference among the aqueous solutions in this respect, the apparent differences among them all approaching or falling within the probable error.

Two per cent aqueous mercurochrome did not affect the permeability of these skins (and in fact, barely stained them on 5 minutes' application); 95% alcohol markedly increased the permeability.

It was noted that the skins treated with the aqueous solutions were much more deeply stained than those immersed in the tincture, despite the differences in iodine contents. This confirms the findings of Karns, Cretcher and Beal,[3] with human skin.

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[3] Karns, G. M., Cretcher, L. H., and Beal, G. D., *J. Pharm. Assn.*, 1932, **21**, 783.

6350

Luteinization of Ovary of Sexually Immature Monkey.*

F. L. HISAW, R. HERTZ, A. HELLBAUM AND H. L. FEVOLD.

From the Zoology Department, University of Wisconsin.

We reported the effects of aqueous pyridine extracts of sheep anterior pituitary on the ovaries of sexually immature *Macacus rhesus* monkeys.[1] We were able to produce marked follicular development in such ovaries, but in no case did definite luteinization occur. This was in agreement with the findings of E. Allen,[2] Hartman,[3] Courrier,[4] Saiki,[5] and Engle.[6]

Nevertheless, intraperitoneal injections of anterior pituitary extracts into adult rats produce a greater degree of luteinization than comparable subcutaneous treatment. Similarly, Friedman[7] showed, in the rabbit, that the type of ovarian response to the urine of pregnant women varies with the method of its administration; ovulation resulted from intravenous injections, whereas intraperitoneal treatment produced only the luteinization of the resulting corpora haemorrhagica. These observations suggested the possibility of altering the ovarian response of sexually immature monkeys by varying the injection procedure. We had previously found that a single intravenous injection of anterior pituitary preparation following numerous subcutaneous injections did not modify the effects obtained. Experiments were consequently devised in which a greater number of intravenous injections were given. We wish to report the response of 2 sexually immature monkeys to such treatment.

Both animals were first given a series of subcutaneous injections of a water soluble fraction of an aqueous pyridine extract of dried sheep pituitary† in doses equal to 0.5 gm. of the dried tissue at

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* Aided in part by grants from the National Research Council, Committee on Problems of Sex, The University of Wisconsin Research Fund, and the Wisconsin Alumni Research Foundation.

[1] Hisaw, F. L., Fevold, H. L., and Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **29**, 204.

[2] Allen, E., *Anat. Rec.*, 1928, **39**, 315.

[3] Hartman, C. G., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 338.

[4] Courrier, R., Kehl, R., Raynaud, R., *C. R. S. de Biol.*, 1929, **101**, 1093.

[5] Saiki, S., *Am. J. Phys.*, 1932, **100**, 8.

[6] Engle, E. T., personal communication.

[7] Friedman, M. H., *Am. J. Phys.*, 1929, **90**, 617.

† The pituitary powder was kindly furnished by the Research Laboratories of Parke, Davis and Company.

12-hour intervals. Monkeys 8 and 9 received respectively 6 and 20 such subcutaneous injections. The initial treatment elicited the cornification of the vaginal epithelium and excessive development of the external genitalia and sexual skin as previously described.[1] At this point laparotomy on monkey 9 revealed ovaries of the following dimensions: right ovary, 2.2x1.8x1.8 cm.; left ovary, 1.9x1.9x1.9 cm. Subsequent findings indicate that the response of monkey 8 was much less extreme and the increased size of the ovaries was obviously proportional to the amount of pituitary material administered. The ovaries were constituted almost exclusively of immense translucent follicles and no signs of luteinization were observed.

Immediately following exploratory laparotomy monkeys 8 and 9 were given respectively 3 and 8 successive daily intravenous injections of 2 gm. equivalents of the same pituitary preparation previously applied subcutaneously. Throughout this period subcutaneous administrations of 0.5 gm. equivalents twice daily were continued. Thirty-six hours after the last injection both ovaries were removed and a biopsy of the uterus taken.

Marked luteinization was observed in the ovaries of Monkey 9, though only a few of the smaller follicles have been completely luteinized. The larger follicles still show clot-filled antra though in most instances the entire granulosa as well as the theca interna have been transformed to characteristic lutein tissue. In numerous cases the luteinized granulosa is separated from the lutein tissue derived from the theca interna by a considerable rim of connective tissue which surrounds the entire follicle.

The ovaries of monkey 8 showed numerous follicles possessing blood-filled antra and luteinized strata granulosa, but in no case had the luteinization extensively involved the theca interna. This limited effect is commensurate with the comparatively brief stimulatory treatment afforded this animal.

The endometrial samples showed clearly that the lutein tissue described above was physiologically functional. The histological picture in both cases is that which obtains in the lutein phase of the normal primate menstrual cycle, though the condition is obviously more exaggerated in the case of the more highly stimulated animal.

Several external influences associated with this induced luteinization were likewise noted. These are chiefly a decrease in redness of the sexual skin, a marked loss of turgidity in the labia majora and sexual skin, and a preponderance of leucocytes in the vaginal smear.

The pituitary preparations employed in these experiments were previously found to possess both follicular stimulating and luteinizing properties when injected subcutaneously into sexually immature rats. These findings show the extraordinarily high threshold of anterior pituitary material required for the luteinization of the primate ovary, and indicate the widely different responses to be obtained from subcutaneous and intravenous injections of the same preparation.

6351

Gonadotropic Action of Phyone on Juvenile Female Rabbit.*

R. HERTZ, A. HELLBAUM AND F. L. HISAW.

Department of Zoology, University of Wisconsin.

Van Dyke and Wallen-Lawrence[1] have prepared an extract of fresh beef pituitary tissue (phyone) possessing marked growth promoting properties on the hypophysectomized as well as on the normal immature rat. This preparation was without effect on the gonads of such test animals. Phyone was consequently thought to contain only the growth promoting hormone of the pituitary gland, and to be free of the sex-stimulating factors known to exist in this gland.

We have assayed 3 different batches of phyone† upon juvenile chinchilla female rabbits from 12 to 13 weeks of age. Four litters of 4 rabbits each were employed. In each litter one rabbit was retained as an uninjected control. Two of the members of each litter were given 0.5 cc. of phyone either intravenously or subcutaneously for from 3 to 5 days. Since Leonard[2] has shown that a female rabbit, if fully adult, will ovulate upon the intravenous injection of phyone, the remaining rabbit in each litter was given a single intravenous injection of 1 cc. of phyone from the batch used on its own litter-mates. This single injection was without effect upon the ovaries of the juvenile rabbits, but 1 cc. samples from any

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* Aided by a grant from the National Research Council, Committee on Problems of Sex.

[1] Van Dyke, H. B., and Wallen-Lawrence, Z., *J. Pharm. and Exp. Ther.*, 1930, **40**, 413.

† This material was obtained from The Wilson Laboratories through the courtesy of Dr. David Klein.

batch invariably produced ovulation upon intravenous injection into an adult rabbit in oestrus. From this difference in response it is apparent that the young rabbits employed here were not sexually mature.

When given repeatedly, as described above, phyone produced marked follicular enlargement and extensive luteinization in the ovaries of the juvenile rabbits. No ovulation occurred, but numerous blood-points were produced. The degree of the response was proportional to the amount of phyone administered, and the route of injection caused no difference in the reaction obtained.

The uteri of all these rabbits reflected the conditions found in their ovaries. Where follicular enlargement predominated, the uterus showed a marked oestrous picture. In those cases where the ovary was more extensively luteinized, the uterus showed a definite pseudopregnant condition. The control uteri in all cases showed only the very slight prooestrous development of the mucosa characteristic of the prepubertal uterus.

It is to be emphasized that each batch of phyone used here was found to be without effect upon the ovaries of sexually immature rats, even when given in the same absolute doses administered to the juvenile rabbits.

On the basis of the negative response of the rat's ovary to phyone, a number of observers have considered this preparation to be free of any of the recognized gonadotropic hormones of the pituitary. Thus, Leonard[2] has presented the possibility of the presence in phyone of a distinct substance causing ovulation in the rabbit. Our findings indicate that such an effect may be due to the gonadotropic content of the phyone employed.

The present work further demonstrates the wide variations in response which may be obtained from different species of test animals with a given tissue extract. It is essential that such differences be checked before attributing a definite hormone content to the preparation being tested.‡

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[2] Leonard, S. L., *Am. J. Physiol.*, 1931, **98**, 406.

‡ Dr. S. L. Leonard, Columbia College of Physicians and Surgeons, has recently favored us with a personal communication confirming these findings on juvenile rabbits.

6352

On the Nature of the Oxygen Linkage in Oxyhemoglobin.

R. D. BARNARD. (Introduced by A. J. Carlson.)

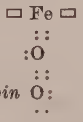
From the Department of Physiology, The Chicago Medical School.

In order to test Haldane's hypothesis that oxygen exists in oxyhemoglobin as a peroxide compound, an attempt was made to identify hydrogen peroxide in solutions of the blood pigment which had been denatured by acid. If oxyhemoglobin is an iron peroxide, we should expect it to behave as do other metallic peroxides, by reacting with acids to form hydrogen peroxide. Inasmuch as the acid hematin resulting from such denaturation has little, if any, catalase activity, any hydrogen peroxide formed should persist in solution.

Solutions of 10 millimolar human oxyhemoglobin were treated with 8 volumes of .04 molar sulphuric acid and then precipitated with 5 volumes of 2% phosphovanadic acid, the latter serving the double purpose of precipitating the protein and of indicating the presence of any peroxide that might be liberated. In every instance the test was negative. Using chromic acid in acetone solution as a test for hydrogen peroxide, none of the latter could be detected in the ordinary Folin-Wu filtrate from blood. The absence of hydrogen peroxide from acid denaturated oxyhemoglobin is deducible from the fact that solutions of benzidine base in acetic acid will not be oxidized to benzidine blue by blood, unless peroxide is added to the system.

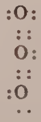
The phosphovanadic acid test described above is sensitive to 1 part of hydrogen peroxide in 200,000. The final dilution attained by the test solution, assuming that all the oxyhemoglobin yielded an equivalent of peroxide, would be 1 millimolar, or 1 part in 30,000.

For this reason the author is inclined to discard the peroxide formula for oxyhemoglobin and to suggest the ozonic linkage. According to this theory, the electronic formula for oxyhemoglobin may be compared with its homologue, ozone:



$$\begin{array}{c} \square\,\mathrm{Fe}\,\square \\ :\,: \\ :\mathrm{O} \\ :\,: \\ \mathrm{O}: \\ \cdot\,\cdot \end{array}$$

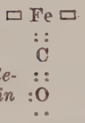
oxyhemoglobin



$$\begin{array}{c} :\mathrm{O}: \\ :\,: \\ \mathrm{O}: \\ :\,: \\ :\mathrm{O} \\ \cdot\,\cdot \end{array}$$

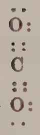
ozone

Carbon monoxide hemoglobin may be represented with its homologue, carbon dioxide, as:



$$\begin{array}{c}\square\,\mathrm{Fe}\,\square\\ ::\\ \mathrm{C}\\ ::\\ :\mathrm{O}\\ \cdot\cdot\end{array}$$

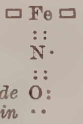
carbon monoxide-hemoglobin



$$\begin{array}{c}\cdot\cdot\\ \mathrm{O}:\\ ::\\ \mathrm{C}\\ ::\\ \mathrm{O}:\\ \cdot\cdot\end{array}$$

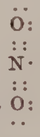
carbon-dioxide

and nitric oxide hemoglobin with nitrogen dioxide:



$$\begin{array}{c}\square\,\mathrm{Fe}\,\square\\ ::\\ \mathrm{N}\cdot\\ ::\\ \mathrm{O}:\\ \cdot\cdot\end{array}$$

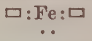
nitric oxide hemoglobin



$$\begin{array}{c}\cdot\cdot\\ \mathrm{O}:\\ ::\\ \mathrm{N}\cdot\\ ::\\ \mathrm{O}:\\ \cdot\cdot\end{array}$$

nitrogen dioxide

It is interesting that molecular oxygen, carbon monoxide and nitric oxide each combine with nascent oxygen, under appropriate conditions, to form the homologues listed. Nascent oxygen could therefore be considered a homologue of reduced hemoglobin:



$$\begin{array}{c}\square:\mathrm{Fe}:\square\\ \cdot\cdot\end{array}$$

reduced hemoglobin

$$\begin{array}{c}:\mathrm{O}:\\ \cdot\cdot\end{array}$$

nascent oxygen

Inasmuch as ozone, carbon dioxide and nitrogen dioxide are hydrated to form acids, it is suggested that the increased acidity of hemoglobin, attendant on its oxygenation or carbonylation, is due to hydration of the resulting compound with the formation of one or 2 additional acidic groups.

6353

Active Immunization in Human Cases with the Polysaccharide of Pneumococcus Type I.

JOSÉ ZOZAYA* AND JANET CLARK.

From the Mulford Biological Laboratories, Sharpe and Dohme, Glenolden, Pa.

Tillett and Francis[1, 2] showed that patients suffering with pneumonia who were injected intracutaneously with small amounts of pneumococcus polysaccharide of the homologous and heterologous types, showed after some time antibodies not only against the infecting type of organism but against the other types. In explanation they suggested that probably the injections of the heterologous polysaccharides produced antibodies.

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* Now at the Gladwyne Research Laboratories, Gladwyne, Pa.

[1] Tillett, W. S., and Francis, T., *J. Exp. Med.*, 1929, **50**, 687.

[2] Francis, T., and Tillett, W. S., *J. Exp. Med.*, 1930, **52**, 573.

Finland and Sutliff[3] confirmed these findings and in addition[4] showed in humans with histories free of recent infection antibody production following the intracutaneous injection of protein-free type specific polysaccharides of either Type I, II, or III Pneumococci.

The present study deals with antibody response following intracutaneous injection of a type specific polysaccharide of Pneumococcus Type I prepared according to the method of Heidelberger, Sia and Kendall.[5] Using the same dose as that used by Tillett and Francis[1] in their work on cutaneous reaction to pneumococcus polysaccharides during lobar pneumonia, 0.1 cc. of a 1:10,000 dilution or 0.01 mg., 10 normal human subjects without recent infection were given 5 weekly intradermal injections of Pneumococcus Type I polysaccharide SSS Pn. I F. The cutaneous reactions varied greatly in intensity in different individuals, positive reactions being elicited in a percentage of these subjects contrary to the findings of the aforementioned workers, who observed positive reaction with very few exceptions only in pneumonia patients at time of crisis and after. Maximum reaction consisted of a bright rash-like erythema 40x50 mm. with pseudopods and itching, appearing within 5 to 15 minutes of injection and followed by marked swelling and tenderness and a central area of redness, reaching its height within 2 hours and gradually diminishing. Intermediate reactions were those of varying degree of erythema and wheal formation and finally clear-cut negative results were observed. There was no apparent relation between these cutaneous reactions and the degree of natural or induced immunity. An irregular variation was also observed in some individuals in the cutaneous reactions following the different injections of the series.

Bleedings were taken prior to injection and one week after the final injection and the serum tested for protective antibodies, precipitins and agglutinins. Subsequent bleeding at monthly intervals were taken from a portion of the group to determine the duration of any acquired immunity.

Homologous antibody response was demonstrated in all cases following the intradermal injections, the serum showing protection against virulent Pneumococcus Type I culture in amounts from 10 M.F.D. to 100,000 M.F.D. This immunity was still demonstrable with little or no decrease 3 months after the final immunizing injection.

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[3] Finland, M., and Sutliff, W. D., *J. Exp. Med.*, 1931, **54**, 637.

[4] Finland, M., and Sutliff, W. D., *J. Exp. Med.*, 1932, **55**, 853.

[5] Heidelberger, M., Sia, R., and Kendall, E. E., *J. Exp. Med.*, 1930, **52**, 477.

No agglutinins were demonstrable in any of the sera. Precipitin reactions with the homologous polysaccharide were obtained, the dilution of the serum giving a reaction corresponding however only roughly to the passive protection titre. No precipitins were found in any of the normal sera showing no natural immunity but were demonstrable in the one case in which natural passive protection was noted.

A summary of the results is given in Table I.

TABLE I.

Immunization of Humans with Pneumococcus Type I Polysaccharide Skin Reaction and Passive Protection.

	Normal Bleeding							Interval Between Last Immunizing Injection and Bleeding							
								1 Week		5 Weeks		9 Weeks		3 Mo.	
Subject	Precipitin Titre	Passive Protection for Mice	Skin Reaction to Weekly Intradermal Injections					Precipitin Titre	Passive Protection M.F.D.	Precipitin Titre	Passive Protection M.F.D.	Precipitin Titre	Passive Protection M.F.D.	Precipitin Titre	Passive Protection M.F.D.
JC	Neg.	Neg.	4	3	3	3	3	Neg.	1000	1:6	1000	1:2	1000	1:2	1000
FE	,,	,,	0	0	0	0	0	,,	1000	1:2	1000				
EB	1:2	1000 M.F.D	2	2	1	2	2	,,	10000						
LM	Neg.	Neg.	3	3	3	3	3	1:6	10000	1:2	100000	1:10	1000	1:2	10000
EE	,,	,,	1	±	±	±	0	Neg.	50	Neg.	100	Neg.	10	Neg.	100
AC	,,	,,	2	3	3	1	1	,,	10						
IG	,,	,,	0	0	0	0	0	,,	1000						
RF	,,	,,	1	±	0	0	0	,,	1000					Neg.	100
RH	,,	,,	0	0	0	0	0	1:2	500						
MP	,,	,,	1	1	±	2	1	1:2	1000						

Precipitin Titre—Serum dilution giving precipitin reaction with SSS Pn I F 1:10000. Read after 4 hours at 37°C. 5° overnight and centrifuged.

Passive Protection for Mice—Number of minimum fatal doses of a 7-hour culture of Pneumococcus Type I against which 0.2 cc. of serum protects mice.

Skin reaction to 0.1 cc. of 1:10000 of SSS Pn I F:

4 —Maximum Reaction—Erythema and swelling 40x40 mm.
3 —Marked Reaction—Erythema and moderate swelling 30x30 mm.
2 —Moderate Reaction—Erythema 20x25 mm.
1 —Slight Reaction—Erythema 10x15 mm.
±—Very slight reaction—Slight erythema 5x10 mm.
0 —No reaction.

6354

Adsorption Isotherms of Some Bacterial Polysaccharides.

JOSÉ ZOZAYA* and LUIS MEDINA.

From the Mulford Biological Laboratories, Sharp and Dohme, Glenolden, Pa.

The study of one of us[1] on the adsorption of bacterial polysaccharides on colloids as antigens suggested further study of the adsorption isotherms of some of these substances, to see if they follow the type of curve usually obtained in adsorption experiments. In the previous work[1] collodion particles, aluminium hydroxide, casein and bacterial cells were used as adsorbents, but in all of these substances with the exception of aluminium hydroxide the adsorption is so slight as to be hardly detectable.

The adsorptive power of charcoal exceeds that of other adsorbents and with this fact in mind as well as the successful immunizing effect which Landsteiner and Jacobs[2] found using this substance as adsorbent with the polysaccharide from *V. cholera,* we used charcoal ("Norit" obtained from A. H. Thomas and Co.) as our adsorbent and as polysaccharides we used that of anthrax, pneumococcus types I and III.

The amounts adsorbed were determined by the precipitin-test with a specific immune serum, titrating the mixed washings from the adsorbent, using as control the same polysaccharide in known concentrations. By comparing the highest dilution of the unknown which gave a definite precipitate with the highest dilution of the control polysaccharide giving the same amount of precipitate, the amount of polysaccharide in the unknown was determined. This method permits a much more accurate determination of the amount of polysaccharide adsorbed than the present chemical method at our disposal (determination of reducing sugars after hydrolysis).

The formula used for the making of the curves is the one given by Freundlich[3]: $a = \alpha c \ 1/n$, where a is the amount adsorbed by 1 gm. of adsorbent, c is the residue in solution of adsorbed substance and α and $1/n$ are constants. This formula is valid at some distance from saturation and can only be applied to the bent rise of the curve. The amounts of carbon used varied from 4.0 to 0.25 gm.

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* Now at the Gladwyne Research Laboratories, Gladwyne, Pa.

[1] Zozaya, J., *J. Exp. Med.*, 1932, **55**, 325.

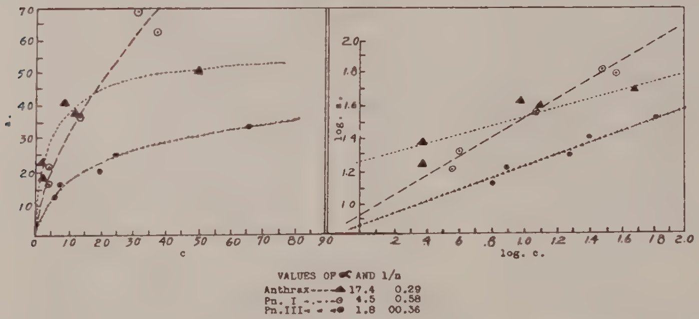
[2] Landsteiner, K., and Jacobs, J., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 570.

[3] Freundlich, H., Colloid and Capillary Chemistry, E. P. Dutton and Co., 1922.

and the polysaccharide from 100 to 10 mg. The volume was kept at 10 cc. We washed the adsorbent with distilled water 6 times. The temperature was that of the room, and the time allowed for adsorption was 30 minutes, keeping the container constantly shaken.

The results of our experiments are given in the graph, in one case plotting the actual values of *a* against *c*, and in the other the logarithms of the same. The values which we found for α and *1/n* of the different polysaccharides are given at the bottom of the graph.

FIG. 1.



Plotted values and curves of a and c values of anthrax, pneumococcus types I and III, polysaccharides adsorbed on charcoal (norit).

The logarithmic curves were all straight lines as was expected and the values of the adsorption exponent *1/n* are all within normal adsorptions. In all these cases the adsorption was of the non-reversible type and since all of the polysaccharides here studied have been successfully tested for antigenicity, it suggests an interesting speculation as to the mechanism of antibody formation with these antigens which will be further discussed in another paper.

Of practical importance is the fact that once the adsorption isotherm is determined and the values of the constants obtained, one can use the equation for calculating the amounts adsorbed from a solution of a given concentration of these substances.

6355

Effects of Intraventricular Injections of Pituitrin and Pilocarpine in Dogs.

JOHN H. LAWRENCE AND DONALD E. DIAL.
(Introduced by Harvey Cushing.)
From the Laboratory for Surgical Research, Harvard Medical School.

Cushing[1] recently showed that the "active principle of the neurohypophysis—pituitrin, when introduced into the ventricle, causes flushing, sweating, salivation, lachrymation, vomiting and pronounced fall in body temperature." This was interpreted as essentially a cranial autonomic or parasympathetic effect, analogous to the reaction on the sympathetic system produced by a discharge of adrenalin into the blood stream. He also found that pilocarpine injected into the ventricle produced essentially the same effect as pituitrin, although less pronounced. When atropine was injected into the ventricle a few minutes before either of the other drugs, or when the patient was under avertin anesthesia, the above reactions did not take place. At Dr. Cushing's suggestion an attempt has been made to compare the reactions thus obtained on human subjects with those of laboratory animals. The present report is based on the results of such studies carried out on dogs.

Methods. In some of the early experiments the animals were under morphia and intravenous chloralose or morphia and ether throughout the experiment. Most of the experiments were carried out with no anesthetic except novocaine infiltration to the skin. Therefore only particularly tractable animals could be used, as the experiments necessitated keeping the animals strapped loosely on the table for several hours.

The animals were first prepared by making, under ether anesthesia, a burr hold in the skull about 1 cm. in diameter in the angle formed by the median bony ridge and the occipital ridge either on one or both sides. A cruciate incision was made in the dura mater and the soft tissues were closed. Two or more days were allowed to elapse before experiments were begun. With a little experience, it was then possible to introduce a blunt lumbar puncture needle into the slit-like lateral ventricle, allow 1 cc. of fluid to flow out and replace it with an equal volume of the drug to be used. The intraventricular injection experiments were controlled by others in which (1) pituitrin or pilocarpine was injected subcutaneously or intramuscularly and (2) the dog was simply kept on the table, either with or without manipulations similar to the ventricular puncture. In only one of the dogs was all of these procedures done; in another the intraventricular injection of pituitrin was done 3 times at 2 day intervals.

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[1] Cushing, H., *Proc. Nat. Acad. Sciences*, 1931, **17**.

Results. Records were kept usually at 15 minute intervals of rectal temperature, pulse, respiratory rate and character, panting, salivation, vomiting, diarrhea and changes in size of the pupils. It was hoped that the observation of the respiratory rate, and especially panting, might be found to be comparable to the sweating found in human beings as a mechanism for lowering body temperature. In this, however, we were disappointed, as panting frequently occurred even before the start of experiments and never seemed to have a definite relation to the injection of drugs. Changes in the pulse rate must also be interpreted cautiously because of the emotional element. The temperature was found to have variations independent of the experimental procedures. Hence the dogs were kept on the table for one or even 2 hours in order to be sure that the temperature had come to a constant level before injections were begun.

The positive results of the work may be summarized as follows:

Group I. Dogs at rest; no anesthesia; no drugs injected. 3 experiments. *Temperature.* Dropped 0.6° to 1.2°C. from the initial level in 2 dogs; rose 0.3° in the third dog; thereafter remained constant with variations of only about 0.1°. In one dog repeated unsuccessful attempts at ventricular puncture were followed by a transient rise of 0.4° followed immediately by a drop to 0.2° above the previous level. *Pulse.* Variations of 25 or more beats per minute either faster or slower but usually faster. *Respirations.* Fairly constant in rate, affected by occasional panting. No salivation, gastrointestinal disturbance or change in size of the pupils.

Group II. Subcutaneous or intramuscular pituitrin, 1 cc.; no anesthesia. 4 experiments. *Temperature.* In 2 dogs varied ±0.2°. In 2 dogs dropped 0.2° and 0.5°, beginning 2 hours and 1½ hours respectively after the injection. *Pulse.* In 3 experiments a drop in rate of 15 to 40 beats per minute lasting one or more hours. *Panting.* Occasional panting in 2 dogs; none in the other 2. *Salivation.* Very slight in one dog. Coryza in one dog.

Group III. Intraventricular Pituitrin, usually 1 cc., with anesthesia. Morphia and chloralose, 5 experiments; morphia and ether, one experiment. *Temperature.* In 2 dogs an immediate drop of about 0.2° within 15 to 30 minutes followed by a rise above the initial level, in one dog of 1.1°, in one dog a drop of 1.4°, not beginning until 2 hours after the injection. In one case the temperature had not been allowed to become stabilized and fell 0.5° further, then rose 1°. In two dogs there was an immediate rise of 0.2° or 0.3°. *Salivation.* In 3 dogs was absent; in 3 was present but slight and occurred only 40 to 70 minutes after the injection. *Diarrhea.* None in 4 dogs; in 1 dog 2 explosive diarrheal movements occurred, 15 and 75 minutes respectively after the injection; in 1 dog an explosive diarrheal movement followed 3 minutes after additional pituitrin, 0.4 cc. was given intravenously.

Group IV. Intraventricular pituitrin usually 1 cc.; no anesthesia or sedative. 8 experiments. *Temperature.* Dropped 0.6° to 0.8° in 5 of 8 experiments (3 on the same dog); in 2 dogs a drop of 0.2° and 0.3° respectively; in one dog which was extremely restless, it rose 1.5°, then fell 0.8°. In practically all cases the drop began within 30 minutes. *Respirations.* Rate increase in all cases. *Panting.* In 7 experiments occurred within 10 minutes and in the 8th in 20 minutes, lasting from 1 to 7 hours. In 2 of these cases the dog had panted at intervals even before the injection. *Salivation.* In 5 cases occurred within 10 minutes; in remaining 3 appeared in 20, 25, and 60 minutes respectively; lasted from 1 to 3 hours. *Vomiting.* Retching in one dog, retching and vomiting in 1 dog. *Diarrhea.* In 3 cases more or less profuse, usually within about 30 minutes, lasting about an hour intermittently.

Group V. Subcutaneous and intramuscular pilocarpine (5 mg. in 1 cc. sterile water); no anesthesia. 3 experiments. *Temperature.* Rise of 0.5° to 1.1° in all 3 dogs starting 15 to 45 minutes after injection. *Pulse.* Rise in pulse rate of 40 to 80 beats per minute. *Panting.* None in 1 dog; moderate in 1 dog; present throughout experiment in 1 dog but more marked after injection. *Salivation.* Profuse, starting in 1 to 7 minutes and lasting 2 or more hours. *Vomiting.* Repeated vomiting in 1 dog, starting 4 minutes after injection and lasting about an hour. *Diarrhea.* Profuse in 2 dogs, starting in 30 minutes; none in third dog.

Group VI. Intraventricular pilocarpine (5 mg.), morphia and chloralose anesthesia. 2 experiments. The data of this group are insufficient for definite conclusions.

Group VII. Intraventricular pilocarpine, (4-5 mg.); no anesthesia. 4 experiments.* *Temperature.* Rise in temperature usually apparent within 30 minutes occurred in all 4 dogs, varying from 0.7° to 2.1°. Striking relation between temperature and respiration curves in that the temperature rose with a drop in respiratory rate. *Respirations and Panting.* Panting absent in 1 case; occasional during first 15 minutes in another; in remainder quite marked and seeming to inhibit the temperature rise. *Salivation.* Profuse in all cases, beginning in from 2 to 6 minutes and continuing for 2 to 5 hours. *Vomiting.* Occurred in one dog 5 minutes and again 8 minutes after injection; one dog vomited during attempt to tap ventricle. *Diarrhea.* In 1 dog 7 minutes after injection.

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* Since this work was finished, Light, Bishop and Kendall. (*J. Pharm. and Exp. Therap.*, 1932, **45**) in this laboratory have produced gastric lesions in rabbits by the intraventricular injection of small amounts of pilocarpine. In our experiments the gastric mucosa was not examined.

Discussion. In comparing the effect of intraventricular pituitrin and pilocarpine on man and dog 2 points must be kept in mind. First, because of differences in the 2 species, it is impossible to compare sweating and flushing, these being very difficult to recognize in the dog. Sweating of the foot pads was looked for but not found. Second, it is very difficult to keep a dog quiet on a table for several hours, and activity on the part of the dog obviously must influence body temperature, respirations, panting, etc. This is borne out by irregularity in results throughout all groups.

Salivation, vomiting and fall in body temperature after intraventricular pituitrin and pilocarpine without anesthesia were more or less consistently present except that pilocarpine caused a rise in temperature rather than a fall.[†] Our hope that the panting of dogs might be found to be comparable to the sweating of human beings was not realized, since panting was so easily evoked by various uncontrollable emotional stimuli. The pulse rate was likewise very labile depending on other factors also, thus interfering with the interpretation of injection effects.

The body temperature was found to be unstable. The room temperature was not carefully regulated or recorded, but the experiments were conducted in a room which was never uncomfortably warm or cold. It was almost the rule that the first temperature taken was a degree or so higher than the level which established itself when the dog had been on the table for some litle time. Unusual struggling or restlessness usually resulted in a rise in temperature as in one dog in Group IV in which we feel inclined to attribute the rise of 1.5° largely to this factor. This may also have contributed to the temperature rise following pilocarpine. The significance of the fall in temperature in the majority of instances after intraventricular pituitrin in unanesthetized dogs is questionable. However, the rise after pilocarpine seems more definite. It is noteworthy that in the one dog in which the intraventricular injection of pituitrin was done 3 times, there was in each case a drop of 0.6° to 0.8°. In 2 dogs in which pituitrin was put into the cisterna magna there was likewise a fall of at least 0.5°.

[†] Light in this laboratory placed 20 mg. of pilocarpine into the ventricles of 2 dogs with rise of temperature to 113 and 113.8°F. respectively with death in 45 minutes.

Salivation was a constant effect of the intraventricular injection of either pituitrin or pilocarpine in unanesthetized animals. The effect was less rapid and less pronounced after pituitrin than after pilocarpine. The pilocarpine seemed to act no more quickly when given intramuscularly than when given intraventricularly or vice versa. In the anesthetized dogs pituitrin caused salivation in only 3 of 6 experiments, was only slight in amount and was slow to make its appearance. Subcutaneous pituitrin caused little or no salivation.

The gastro-intestinal effects were not pronounced. Retching or vomiting was only occasional. In 3 of 8 instances intraventricular pituitrin was followed by more or less active catharsis which is possibly of significance.

Cushing has shown that rectal avertin anesthesia inhibits the effect of pituitrin given into the ventricle. The inconstant and irregular results obtained here in a limited number of experiments under chloralose anesthesia although not conclusive suggest that the same is true for chloralose. While it is possible as we have shown to perform experiments such as here described without using any anesthesia whatever, the results are so easily influenced by many external factors that an anesthetic which will not block the centers in the neighborhood of the third ventricle, if such an anesthetic exists, is desirable if reliable results are to be obtained with dogs.

Summary. Pituitrin and pilocarpine have been administered to dogs by various routes and under various conditions. Intraventricular pituitrin in unanesthetized animals resulted in a fall in body temperature in the majority of cases, usually 0.6° or more, in distinct salivation, and in several instances in stimulation of the smooth muscle of the gastro-intestinal tract resulting in retching, vomiting or evacuation of the bowel. In animals anesthetized with chloralose there was less regularity in the effects produced, possibly because the anesthetic acts on the same centers as pituitrin.

Pilocarpine intraventricularly in unanesthetized dogs resulted in a rise in temperature and profuse salivation as did intramuscular pilocarpine. Gastro-intestinal effects occurred more frequently after intramuscular and subcutaneous pilocarpine than after intraventricular injection.

When these results are compared with those on man, it is evident that they are less conclusive and reveal marked differences in the reactions of man and dog to the effect of pituitrin and pilocarpine when introduced into the ventricles.

6356

Effect of Sympathomimetic and Parasympathomimetic Drugs on Motility of the Gastro-Intestinal Tract of Elasmobranch Fishes.

J. V. V. NICHOLLS. (Introduced by B. P. Babkin.)

From the Atlantic Biological Station, St. Andrews, N. B., and the Department of Physiology, McGill University.

It has been demonstrated (Nicholls[1]) that excised strips of the stomach of *Raja diaphanes* and *erinacea* suspended in a nutrient solution (NaCl, 16.38 gm.; urea, 21.6 gm.; KCl, 0.894 gm.; $CaCl_2$, 1.110 gm.; $NaHCO_3$, 0.378 gm.; NaH_2PO_4, 0.06 gm.; and dextrose, 1.0 gm. per litre of distilled water; with a pH of 7.8) will react to adrenalin, pilocarpin and acetyl-cholin.

Adrenalin (1:100,000 to 1:250,000) stimulates all parts of the stomach, raising the base-line rate and amplitude of the contractions. The only exception is in the antral region near the pyloric canal, where it stimulates in concentrations of 1:2,000,000, but inhibits in concentrations greater than 1:1,000,000. Adrenalin following pilocarpin or acetyl-cholin has an additive effect. Pilocarpin (1:250,000) and acetyl-cholin (1:100,000) stimulate all parts of the stomach. The former increases the rate and amplitude of the contractions, whereas the latter raises the base-line as well as increasing the rate and amplitude. Atropin (1:250,000) has no effect on the spontaneous contractions, but counteracts the effect of acetyl-cholin and pilocarpin, restoring the contractions to normal.

Thus it was shown that adrenalin not only stimulates isolated strips of the stomach (Dreyer[2] and Lutz[3]), but it has a definite inhibitory action, due probably to the hypersensitivity of a special region of the stomach to adrenalin.

The well marked effect of adrenalin, pilocarpin and acetyl-cholin lends further proof to the fact, first discovered by Bottazzi,[4] that there is a well developed motor sympathetic and parasympathetic nerve supply to the stomach of elasmobranch fishes.

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[1] Nicholls, J. V. V., *Contributions to Canadian Biology and Fisheries*, in press.

[2] Dreyer, N. B., *Trans. Nova Scotia Inst. Sci.*, 1928, **17**, 199.

[3] Lutz, B. R., *Biol. Bull.*, 1931, **61**, 93.

[4] Bottazzi, F., *Z. f. Biol.*, 1902, **43**, 372.

The effect of these drugs on the spiral intestine and valve, colon and rectum was also studied and the results tabulated:

TABLE I.

Region	Adrenalin (1:250,000)	Acetyl-cholin (1:100,000)	Pilocarpin (1:250,000)
I. Spiral Intestine:			
(1) Duodenum	stimulates	stimulates	stimulates
(2) Lower end	”	”	”
II. Spiral Valve:			
(1) Duodenum	”	”	doubtful effect
(2) Lower end	”	”	” ”
III. Colon	”	”	stimulates
IV. Rectum	”	”	”

Adrenalin stimulates all these parts, raising the base-line, rate and amplitude of the contractions. However, on two occasions indications of inhibition were found in the spiral intestine, the rate and amplitude of the contractions being lowered. Adrenalin following pilocarpin or acetyl-cholin has an additive effect on all the above parts. These results do not agree with those of Lutz,[3] who found that adrenalin caused a decrease in the tone and inhibition of the motility of the posterior end of the intestine and rectum. Acetyl-cholin stimulates all these parts, raising the base-line, rate and amplitude of the contractions. Pilocarpin acts similarly on all parts except the spiral valve where the effect is doubtful, there being no change in the rate or base-line, though the amplitude of the contractions is usually slightly increased.

Our results confirm those of Bottazzi[4] that stimulation of the vagus activated the gastro-intestinal tract down to the lower end of the spiral intestine, and that stimulation of the 45th to 48th segments of the spinal cord activated the rectum and colon.

Finally, experiments were carried out on the rates of contraction of various parts of the gastro-intestinal tract at different temperatures. At any one temperature different parts of the stomach have different rates of contraction. The parts named in order of decreasing rate are: pylorus, fundic and antral regions of the lesser curvature, fundic region of the greater curvature, antral region of the greater curvature and cardia. The upper and lower ends of the spiral intestine were found to have the same rate of contraction, whereas the upper end of the spiral valve had a more rapid rate than the lower end. In the colon and rectum no rhythmic contractions could be obtained, merely spontaneous spasmodic contractions at intervals of about one hour. It seems that a gradient of excitability (Alvarez[5]) exists in the spiral valve but not in the spiral intestine, the reason being probably that the spiral valve is mainly responsible for the movement of the contents, while the spiral intestine acts merely as a churning mechanism.

The fact that the colon and rectum only contract spasmodically, are very distensible, and are sensitive to mechanical stimulation such as stretching, would seem to show that their function is that of a reservoir which empties itself from time to time automatically or on distention, their activity being regulated in some way by both divisions of the autonomic nervous system.

6357

Experimental Endometrial Embolism in the Rabbit.

VICTOR C. JACOBSEN.

From the Department of Pathology, Albany Medical College.

The fate of autologous endometrial tissue introduced into the venous circulation was studied in 11 rabbits in the "resting" stage, "in heat," post-partum and also in pregnancy. The operation consisted of laparatomy, with removal of about 1 cm. of a cornu, curettage of this portion, suspension of the scrapings in 3 cc. of homologous blood serum at room temperature, and immediate injection into the rabbit's ear vein. Histologic study of the tissues was made 30 sec., 12 hr., 24 hr., 48 hr., 1 week, 1 month, 69 days, 79 days, 109 days, and 130 days, after operation, the animals except for those which died of "embolic shock" 30 seconds after the injection, remaining in good health the entire period of their survival. The bulk of the cellular material was apparently filtered out in the lung arterioles and capillaries, no adventitious cells being found in the heart, spleen, kidneys or liver. Embolic cells, apparently viable could be recognized one week after injection but none could be identified after that time. Stromal cells possibly survive longer than epithelium. Fibrin is deposited about the cell emboli, their cytoplasm gradually disappears, the nuclei become pyknotic and break up, the embolus becoming converted into a hyaline mass in the smaller vessels and organized in the larger arterioles. Complete absorption or autolysis results in clearing of the pulmonary vessels of the autologous cell masses with no suggestion of formation of new endometrial tissue. These results are in agreement with the distribution of the characteristic lesions in cases of human ectopic endometriosis. While Sampson[1] has found a venous dissemination of endometrial tissue within the human uterus and possibly in the groin, no instance of metastasis of non-neoplastic endometrial emboli has yet been observed above the diaphragm.

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[5] Alvarez, W. C., "The Mechanics of the Digestive Tract", 2nd ed., 1928, 55.

Halban[2] invoked lymphatic metastasis from the uterus to explain ectopic pelvic endometriosis, especially its extraperitoneal locations. He had recognized characteristic uterine glands in the regional lymph nodes. Glands have been found in pelvic lymph nodes in both the male and female but without typical endometrial stroma or menstrual reaction. However, since a venous invasion has been proved a lymphatic dissemination is at least theoretically possible.

6358

Effect of Parathyroid Hormone and Sodium Acid Phosphate on Bones of Guinea Pigs.*

ROBERT C. GRAUER. (Introduced by Samuel R. Haythorn.)

From the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.

When parathyroid hormone is administered to normal subjects there is a rise in the serum calcium and a decrease in the serum phosphorus. Albright and Ellsworth believed that, following the administration of parathyroid hormone, the body fluids became depleted of phosphorus as evidenced by falling serum phosphorus; the tendency to make up the deficiency of the phosphorus and consequently the phosphate ions in the blood serum was met by removing calcium phosphate from the bones and a rise in the serum calcium ensued.

The recognition of the relationship of decalcification of bone in clinical hyperparathyroidism (fibrous osteodystrophy) and the experimental production of similar lesions by the use of the hormone of the parathyroid gland have interested us in the study of the effect of simultaneous injections of phosphate and parathyroid hormone. Three groups of growing guinea pigs whose average weight was 215 gm. were employed. One group of 3 animals received increasing daily doses of parathyroid hormone beginning with 5 units and were injected for 25 days. The second group received daily increasing doses of $Na_2HPO_4.12H_2O$ in aqueous solution so that 1 cc. of the solution equaled 0.075 gm. of Na_2HPO_4 or 0.05 gm. of PO_4 or 0.0163 gm. of P. The injections were made daily into the peritoneal cavities, beginning with ¼ cc. and gradually increasing the dose to 1 cc. Preliminary injections had shown that a dose of over 1 cc. was rapidly fatal. The third group received subcutaneous injections of parathyroid hormone and intraperitoneal injections of the phosphate solution simultaneously. The doses were given in the same amount as that administered to the control groups. The diet allowed the animals was the same as that given our regular stock guinea pigs and consisted of white bread, leafy greens, hay and oats.

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[1] Sampson, J. A., *Am. J. Path.*, 1927, **3**, 93.

[2] Halban, J., *Wien. klin. Wchnschr.*, 1924, **37**, 1205.

* The parathyroid hormone employed in this experiment was kindly supplied by the E. R. Squibb Co.

The combined action of parathyroid hormone and sodium acid phosphate was considerably more toxic to the guinea pigs than either the parathyroid or phosphate solutions alone. The injection of 5 units of parathyroid and ¼ cc. of phosphate produced a mild transient tetany that lasted for a few minutes, while the control groups were symptomless. Tetany was observed in the control animals only when the dose of phosphate injected alone was over ½ cc. The toxicity became more pronounced with the advance of time so that the animals receiving phosphate plus parathyroid hormone were either found dead or were *in extremis* at the end of 19, 20, and 22 days respectively. The total amount of parathyroid hormone administered to the control animals varied from 250 units to 370 units. The total amount of phosphate and parathyroid hormone given to the group which received simultaneous injections varied from 220 units of parathyroid to 310 units and from 11 cc. to 14 cc. of phosphate solution. Sections were taken from all the bones and from the soft tissues. They were fixed in Zenker's fluid, decalcified with 2% nitric acid and were stained with phloxine and methylene blue.

We wished to observe the toxic effects of the phosphate solution on an animal that had received parathyroid for a length of time. One control animal, after having received daily injections of parathyroid hormone for 23 days, was given 2 intraperitoneal injections of ½ cc. each of phosphate solution on the 24th and 25th days respectively. This guinea pig showed marked weakness of the extremities on the morning of the second day and was found dead a few hours later.

Microscopic studies of the long bones and costochondral junctions of the ribs revealed marked differences in the degree of decalcification in the respective groups. The control parathyroid guinea pigs showed osteoclastic activity and fibrous replacement at the costochondral junctions with a minimal amount of lacunar resorption in the corticalis. The long bones showed the greatest amount of osteoclasis and fibrous ingrowth, though this was pronounced, at the growing ends of the bones with lesser lacunar resorption and fibrous replacement in the shaft.

Those animals that received the phosphate solution in addition to parathyroid hormone showed a greater degree of fibrous replacement in the shafts of the long bones, in the marrow cavity, and more active osteoclasis throughout. It required twice the amount of parathyroid hormone alone to produce the degree of decalcification and other pathologic changes observed in fibrous osteodystrophy *(Osteitis fibrosa cystica)* than when parathyroid and the phosphate solution were used together. All the sections in this group showed wide hemorrhagic areas in the marrow cavities of the long bones.

6359

Anterior Pituitary Therapy and Uterine Motility in the Unanesthetized Rabbit.

SAMUEL R. M. REYNOLDS.* (Introduced by Ferdinand C. Lee.)

From the Department of Embryology, Carnegie Institution of Washington, Baltimore.

In earlier experiments[1, 2] on the action of the ovary-stimulating substance of human urine of pregnancy on uterine motility, it was shown that the contracting uterus of the unanesthetized rabbit either approaches the quiescent state or becomes fully quiescent 5 to 7 hours following a single intravenous injection of this material. This takes place whether or not the ovaries are present and so may happen independently of ovarian tissues which might contribute to the effect. The significance of this finding has not been determined however, and it has never been shown that the action of urine-substance parallels the action of anterior pituitary therapy in its effect on uterine motility as it does in certain respects upon the ovary. The data from the present experiments give this latter information and show that the effect of the injection of fresh saline suspension of anterior pituitary glands of the ox or the injection of alkaline extracts of these glands exactly parallels the effect of the injection of pregnancy urine-substance.

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* Fellow in Medicine, National Research Council, 1931-32.

[1] Reynolds, S. R. M., *Am. J. Physiol.*, 1932, **100**, 545.

[2] Reynolds, S. R. M., and Friedman, M. H., *Am. J. Physiol.*, 1930, **94**, 705.

Beef anterior pituitary preparations used were of 2 varieties: (1) Fresh saline suspension was made within 4 hours of the death of the animal by triturating the glands in a mortar and using the supernatant fluid either immediately or after standing for 2 to 3 days in the ice box. The amount employed was the fluid obtained from 1 to 3 glands. This was at least 5 to 15 times the m. e. d. for ovulation in a *post partum* rabbit of medium weight. (2) An alkaline extract of ox pituitaries similar to that described by Bugbee *et al.*[3] was also used. Since the extracts were not freshly made when employed, the approximate m. e. d. for ovulation was determined at the time each extract was used. 0.1-0.2 cc. in a single intravenous injection regularly elicited ovulation. The amounts used in the experiments, however, ranged from 10 to 30 times this amount (equivalent to ½-1½ gm. fresh tissue).

TABLE I.

Condition of Rabbits	Response	Partial Response	No Response	Total
Intact (fistula)	5	0	0	5
Castrated (fistula)	5	4	1	10
Total	10	4	1	15

Since the technique of these experiments exactly duplicates others already described,[1] a mere summary of the results is given in Table I. A "response" means that within 5 to 7 hours a decrease of the spontaneous rhythmical motility occurred, so that nearly complete quiescence supervened, in intact unanesthetized rabbits; in castrated rabbits the "response" refers to a similar, but transitory quiescence of motility which has been induced by oestrin (Theelin). A "partial response" indicates that the quiescent condition was approximated, but not attained within the time that the motility was recorded. The possible reasons for this have been discussed before,[1] as have the necessary precautions in this type of experimentation.

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[3] Bugbee, E. P., Simond, A. E., and Grimes, H. M., *Endocrinology*, 1931, **15**, 41.

Two experiments in which inactivated (boiled) extracts were used showed that extracts so treated were without effect on uterine motility. Therefore, the "response" we have obtained is probably specific for active extracts. The significance of these results may possibly be indicated by the close similarity in action of anterior lobe therapy and of human pregnancy-urine, but it may be no more than a coincidence that quiescence of uterine motility normally takes place following coitus and prior to ovulation in the intact animal[4] and under the conditions just described.

6360

Suprarenal Cortex and Temperature Regulation.

LELAND C. WYMAN AND CAROLINE TUM SUDEN.
(Introduced by F. H. Pratt.)

From the Department of Physiology, Boston University School of Medicine, and the Evans Memorial, Massachusetts Memorial Hospitals.

The presence of the suprarenal cortex is necessary for proper temperature regulation in the rat. Wyman and tum Suden[1] found that suprarenalectomized rats having gross masses of cortical tissue, transplanted or accessory, maintained their body temperature as well as normal rats during 2 hours in a moderately cold room (40° to 50° F), but that rats having cortical insufficiency suffered a decline in body temperature and did not recover their original temperatures in a warm room so soon as those having cortical tissue. It was suggested that a disturbance in physical heat regulation, i. e., increased heat loss, as well as metabolic disturbance might account for this disability. Hartman, Brownell and Crosby[2] found that the administration of cortin enabled suprarenalectomized rats to produce heat and maintain their temperature in a cold environment almost as well as normals, and suggested that inadequate heat production is responsible for the disability of suprarenalectomized animals.

The importance of the sympathico-adrenal system in temperature regulation is well known. Cannon, Querido, Britton and Bright[3] concluded that excitation of the suprarenal medulla constitutes a primary line of defense when there is a disturbing heat loss, the extra output of adrenin increasing heat production, while a secondary line of defense would be increased muscular activity, i. e., shivering. Hartman, Brownell and Crosby[2] pointed out that shivering is a weak reaction in the rat as compared with other animals, and suggested that the reduction in voluntary muscular activity seen in suprarenalectomized rats, especially in a cold environment, might in part explain their inability to maintain their temperature when threatened with heat loss. The suprarenal cortex, therefore, would be an important part of the secondary line of defense.

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[4] Reynolds, S. R. M., and Friedman, M. H., *Am. J. Physiol.*, 1930, **94**, 696.

[1] Wyman, L. C., and tum Suden, C., *Am. J. Physiol.*, 1929, **89**, 362.

[2] Hartman, F. A., Brownell, K. A., and Crosby, A. A., *Am. J. Physiol.*, 1931, **98**, 674.

Inasmuch as Wyman and tum Suden could not detect any severe disability in suprarenalectomized rats with transplanted cortical tissue but no demonstrable medullary tissue, in the moderately cold environments used, they suggested that a critical temperature might be found at which, lack of the medulla in rats would result in difficulty in temperature regulation. It was thought that exposing the animal suddenly to a very low temperature in a restricted space might throw some light on the subject. This has been done with 5 normal rats, 5 rats with one suprarenal removed and from one-half to three-quarters of the other resected, and 6 suprarenalectomized rats having transplanted cortical tissue but no medullary tissue. Three of the latter group were tested twice. All were males, operations performed 71 to 76 days of age, and temperature experiments 112 to 142 days after operation. The control rats were of the same age as the experimental animals. Rectal temperatures were taken with a certified clinical thermometer. Exposure to cold was carried out in a small tin box surrounded by a cooling mixture (ice and $CaCl_2$). Three temperature readings were taken, one at room temperature, one after one hour in the cold box, and again after one hour at room temperature, to test recovery. The results are given in table 1. These results were practically the same for all 3 types of rat with respect to fall of temperature in the cold-box and recovery after removal to room temperature. The marked decline in body temperature at the lower temperatures and the falls greater than those previously observed at the higher temperatures may be accounted for by the facts that the rat is not a perfectly homoiothermic animal, and that the small size of the cold box restricted voluntary muscular activity. This further emphasizes the suggestion

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[3] Cannon, W. B., Querido, A., Britton, S. W., and Bright, E. M., *Am. J. Physiol.*, 1927, **79**, 466.

TABLE I
Temperature change after 1 hour in cold box.

Type of Rat	No. of cases	Body temp. at room temp.	Temp. of cold box	Fall in body temp. in cold box	Diff. from original body temp. after 1 hr. at room temp.
		°F.	°C.	°F.	°F.
Control	2	98.0, 98.4	4, 5	3.6, 5.9	—1.0, +1.4
Partial suprarenalect.	1	96.5	5	3.5	+1.7
Transplant	6	97 to 99.2	4 to 10	0 to 5.2	
Normal	3	97.4 to 98.4	—6 to —9	7 to 7.6	—0.2 to —3.3
Partial suprarenalect.	4	96.6 to 99.8	—5 to —10	6.6 to 8.8	+2.2 to —1.8
Transplant	3	97.8 to 98.4	—3 to —10	6 to 8	0 to —2.2

made by Hartman[2] with regard to the importance of voluntary activity in the rat.

The absence of any difference in behavior with regard to the maintenance of body temperature between normal rats and those lacking the suprarenal medulla but having cortical tissue, in this and in previous researches, indicates that evidence of the operation of the primary line of defense, *i. e.,* medulli-adrenal secretion, in heat loss must be sought by other means. It is possible that loss of the medulla is compensated for by the activity of the rest of the sympathico-adrenal system in heat regulation in this animal. The importance of the suprarenal cortex in the secondary line of defense appears to be well established.

6361

Cholesterol Ester Percentage in Diabetic Plasma.*

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Since the advent of insulin there has been a marked change in the relative causes of death in diabetics. Whereas formerly 15% of the patients died from arteriosclerosis and 60% from coma, now 47% die from arteriosclerosis and only 20% from coma. Insulin has rescued the diabetic from death by coma only to have him die from arteriosclerosis.[1] Concerning non-diabetic arteriosclerosis, Aschoff[2] believes that an increase in the cholesterol content of the plasma is the initial step in its production. He states that with increased amounts of cholesterol more and more deposition takes place, mainly in the form of cholesterol esters. These esters are then broken down, the cholesterol crystallizing out and the fatty acid combining with calcium to form soaps which later are changed over into inorganic salts. Schönheimer[3] finds that in sclerotic arteries there is a deposition of cholesterol much as it is in the blood, i. e., 50-60% esterified and 25-26% free, and that this ratio remains constant. Since he finds that the ester content of calcified arteries is as high as in the uncalcified, he is not in agreement with Aschoff that esters change to calcium salts. Knudson found that feeding cholesterol or cholesterol esters with a fat-free diet resulted in increased total cholesterol but no increase in esterified cholesterol.[4] On the other hand feeding fat without cholesterol resulted in no increase of total cholesterol but an increase in esterified cholesterol,[5] that is, cholesterol takes part in fatty acid absorption by the formation of cholesterol esters. Bloor and Knudson[6] found in plasma of unselected diabetics that there was no increase in the percentages of cholesterol esters even though the total cholesterol was in some cases 4-5 times normal.

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* This work was supported by a fund for the study of diabetes donated by Mr. William H. Robinson of Pittsburgh.

However, diabetic patients with the most advanced arteriosclerotic changes might show the most marked changes in cholesterol. Because of the evident importance of cholesterol esters in arteriosclerosis the percentages of these substances were studied. The methods used were the colorimetric procedure of Bloor and Knudson and for comparison the Windaus digitonin precipitation method as adapted by Okey.[7] It may be said that while the colorimetric values obtained were about 25-30% higher, the ratio of free to bound cholesterol is practically the same by the 2 methods. Fifteen patients from the Diabetic Clinic of this medical school were selected.

Group A consisted of 9 patients with such severe arteriosclerotic changes in the lower extremities that gangrene had resulted and rendered necessary amputation of a toe, foot or leg. The duration of the diabetes varied from 1 to 18 years, the ages from 42 to 75 years. Five were of sufficient diabetic severity as to require insulin. The diets were at low maintenance levels and the percentage of fat calories in the diets averaged 58%.

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[1] Joslin, Treatment of Diabetes, 1928.

[2] Aschoff, L., *Beiheft z. Med. Klinik,* 1908, 1.

[3] Schönheimer, R., *Zts. f. Physiol. Chem.,* 1926, **160**, 61.

[4] Knudson, A., *J. Biol. Chem.,* 1921, **45**, 255.

[5] Knudson, A., *J. Biol. Chem.,* 1917, **32**, 337.

[6] Bloor, W. R., and Knudson, A., *J. Biol. Chem.,* 1917, **29**, 7.

[7] Okey, R., *J. Biol. Chem.,* 1930, **88**, 367.

Group B was composed of 6 patients with advanced arteriosclerosis in the lower extremities showing evidence of impending circulatory insufficiency but not having actual gangrene. In these, diabetes had existed from 1.5 to 19 years and the ages ranged from 38 to 70 years. All but one required insulin for diabetic control. Diets were low maintenance and the percentage of fat calories averaged 62%.

For controls, 12 normal individuals with an average age of about 32 years were studied.

In all instances samples of blood were obtained after an overnight fast or after a fat-free breakast.

TABLE I.
Average of Results.

	No. Cases	No. det.	Digitonin Method			Colorimetric Method			Blood Sugar	% Fat Cal. in Diet	Av. Age
			Total	Ester	Ester %	Total	Ester	Ester %			
Group A	9	34	229	158	69	293	216	72	181	58	60
Group B	6	15	199	148	70	250	186	75	163	62	65
Normals	12	12				235	147	62		34	32
Normals K & B	26	26				200	116	58		34	

Summary: The total and esterified cholesterol values were found to reach their highest levels in the group with the most advanced sclerosis (Group A). The percentage of esterified cholesterol in both groups with arteriosclerotic changes was found to be 10-15% above that of normal averages. These results suggest a probable relationship of high percentage of cholesterol-ester in blood plama to the development of athermatous changes in diabetic patients. The possible relationship of the high percentage of cholesterol esters in these patients to the high fat diet is not excluded and is now being investigated.

6362

Seasonal Variation of Antirachitic Effect of Sunshine in the Latitude 42° 39′ (Albany, N. Y.)

ARTHUR KNUDSON.

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In the course of studies on the comparative value of various light sources for the cure and prevention of rickets[1] a study was also undertaken to determine the seasonal antirachitic efficiency of sunshine in latitude 42° 39′ (Albany). These experiments have been continued over a period of 2½ years.

Rats at 4 weeks of age were put on the Steenbock rachitic diet 2965 and both the curative and preventive type of experiments were used. In the curative type of experiment the animals, having developed a severe degree of rickets within 21 days, were exposed to sunshine for varying lengths of time between 10 A.M. and 3 P.M. in the winter months and between 11 A.M. and 1 P.M. in the summer months. Treatment was continued for 21 days. In the preventive type of experiment the animals were exposed to the sun's rays for a period of 6 weeks from the time that they were put on the rachitic diet. Skyshine as well as sunshine was available in these experiments. No attempt was made to compensate for the cloudy days so that on cloudy days rats received skylight or reflected light from the clouds and sky. Several different dosages were tried in each type of experiment in order to determine the length of exposure required either to bring about complete healing of rickets within 3 weeks or to prevent the development of rickets. Diagnosis was based on roentgenograms. Along with these observations determinations of the ash in the dry fat-free extracted femurs were made and also in most instances the blood phosphorus.

In the curative experiments exposures of 240 to 270 minutes per day during the months of December and January showed very slight or no healing. In February, particularly the latter half, there is noted an increase in healing but it is not until April that 270 minutes exposure per day shows complete healing. The months of June, July and August are the most effective periods of the year and it was found that 90 to 120 minutes exposure per day gave complete healing. September, October, and November show gradual falling off of antirachitic effect but beginning about December first there is a very decided decrease.

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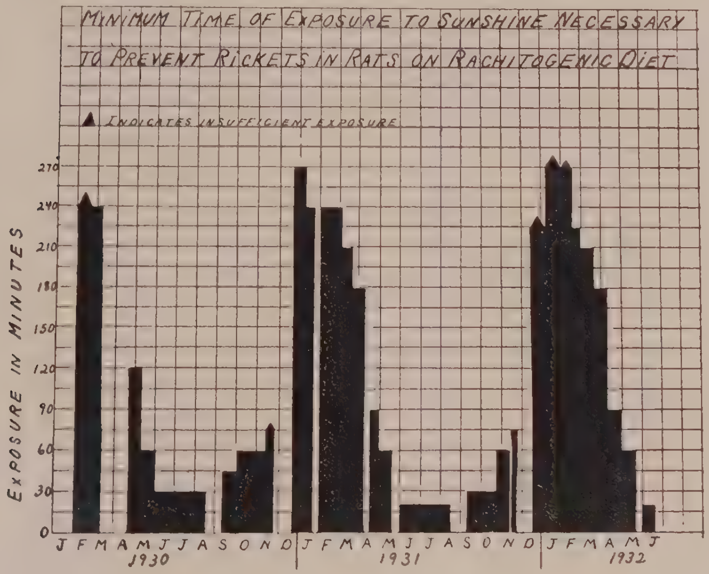
[1] **Knudson, Arthur,** *Am. J. Dis. Children,* **1932, 44, 531.**



The preventive type of experiments show the variations throughout the year more strikingly than do the curative experiments. From the roentgenograms taken at intervals of 3 weeks the minimum length of exposure necessary to prevent rickets has been plotted and is shown on the accompanying chart. In this chart there are certain gaps, particularly in 1930, in which there were no experiments covering those periods. The year 1931 and 1932 up to June 20th are however more complete. In the latter half of December and the months of January and February 240 to 270 minutes exposure per day is just about sufficient to afford complete protection. There is some increase in antirachitic efficiency March first, but a much greater increase occurs around April first. June, July, and August require an exposure of only 20 minutes per day. The antirachitic effect of midsummer sunshine is therefore about 12 to 14 times that of midwinter sunshine. September, October and November show gradual decreases in antirachitic action of sunshine. In December as in the case of observations on curative experiments, there is a sudden drop in antirachitic activity.

Since very little radiation is effective through the hair and as has been show previously[2] exposure of about one-eightieth of the surface area of the skin of the rat produces greater healing of rickets than of the whole animal, it may be inferred that perhaps the above data might be applied to humans. One-eightieth of the surface area in the human would always be less than the area of the face and hands. It should be possible to obtain ample radiation in this vicinity for the prevention of rickets for at least 8 to 10 months of the year by exposure of the face and hands to sunshine.

6363

Effects of the Introduction of Blood from Bred Rabbits upon Immature Rabbits.*

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The purpose of this study was to demonstrate the occurrence of anterior lobe hormone in the blood of the bred rabbit during the time elapsing between coitus and ovulation.

In the first series of experiments 24 rabbits were bled to death at times varying between 1½ and 12 hours post coitum. The serum varying in amounts between 20 and 60 cc. was injected into immature females. One animal that had received 60 cc. of serum from a donor killed 1½ hours post-coitum responded positively.

In the second series, the donors were killed between 1½ and 2 hours post-coitum, the blood defibrinated and immediately introduced intravenously into immature females which had first been bled of part of their own blood. Five of the 12 animals used

TABLE I.

Post Copulation Time	Blood Removed from Recip't	Blood Int'd from Donor	Time Killed Post-Injection	Results
Hr.	cc.	cc.	hr.	
1¾	50	95	51	Large Corp. Hemm.
1¾	55	100	51	" " "
1½	60	95	52	Corpora Lutea.
1¾	45	75	48	Large Corp. Hemm.
1¾	50	100	48	" " "

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[2] Knudson, Arthur, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 314.

* This investigation was aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

responded positively, either large corpora hemorrhagica or corpora lutea resulting. Controls given corresponding amounts of blood from unbred females or from males failed to respond. The results of the positive experiments are tabulated.

Urine collected from bred animals at periods of 8 and 12 hours post-coitum gave no response in immature rats.

Extracts of blood serum obtained by fractional precipitation with sodium sulfate from donors killed at varying times post-coitum and injected into immature rats in amounts equivalent to 50 cc. of blood serum gave no response.

Conclusions. Some evidence was obtained supporting the theory that ovulation in the rabbit occurs as a result of an increased production of anterior lobe hormone.

6364

Coprophagy as a Source of Vitamin B (B_1).

L. E. BOOHER AND T. KANEKO. (Introduced by H. C. Sherman.)

From the Department of Chemistry, Columbia University.

Many experimental investigations with animals on simplified food mixtures have indicated that the consumption of their own feces contributes something nutritionally beneficial, due either to unassimilated food residues or to a synthesis by bacteria in the intestinal tract. The report of Fridericia and coworkers[1] on the phenomenon which they termed "refection," whereby young rats deprived of the vitamin B-complex may recover "spontaneously" from the effects of this deficiency and may transmit this immunity to the need for vitamin B in the diet to other rats has been confirmed by several other investigators.[2, 3, 4, 5] According to these investigators the presence of a large proportion of *raw* starch in the vitamin B-deficient diet is an essential condition for the occurrence of refection. Mendel and Vickery[6] were unable to secure refection when they attempted to reproduce the conditions described by the European investigators. Steenbock, Sell and Nelson,[7] using diets deficient in vitamin B and containing a large proportion of dextrin rather than raw starch, considered that the beneficial effects of coprophagy in their rats receiving inadequate addenda of vitamin B were due to unabsorbed vitamin B, since in the absence of vitamin B addenda coprophagy did not prolong life. Roscoe[4] studied the effects of coprophagy with young rats deprived of vitamin B, or vitamin B_1 and B_4. When the diet contained raw rice starch the animals were not benefited by coprophagy unless they chanced to have become refected. If this same diet was steamed for 3-4 hours, the animals did not become refected but could grow at a fairly good rate provided a *large proportion* of the excreted feces was consumed by them.

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[1] Fridericia, L. S., *et al.*, *J. Hyg.*, 1927, **27**, 70.

[2] Roscoe, M. H., *J. Hyg.*, 1927, **27**, 103.

[3] Kon, S. K., and Watchorn, E., *J. Hyg.*, 1928, **27**, 321.

[4] Roscoe, M. H., *Biochem. J.*, 1931, **25**, 2056.

[5] Kon, S. K., *J. Hyg.*, 1931, **31**, 543.

[6] Mendel, L. B., and Vickery, H. V., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 552.

The present investigation was undertaken to ascertain to what extent our vitamin B (B_1) studies may be expected to be influenced by the interfering factor of coprophagy. Young albino rats reared to 28 days of age by mothers whose diets consisted principally of milk powder and ground whole wheat were caged on raised mesh screens and were fed the vitamin B(B_1) deficient diet previously described by Sherman and Chase.[8] This diet consists of casein (extracted) 18%, Osborne and Mendel salt mixture 4%, butterfat 8%, cod-liver oil 2%, autoclaved baker's yeast 15%, and *raw* cornstarch (Duryea) 53%. When the animals had reached stationary weight, which occurred regularly after 12 to 16 days, the animals of each litter were divided into 2 groups, matched as to size and sex and transferred to individual cages with raised mesh screens.

One group was continued on the vitamin B(B_1) deficient diet without supplement and all died within the usual time, 24 to 33 days beyond the time at which they had reached stationary weight. In several instances animals of this group were known to have acquired and consumed small quantities of their feces but in all cases the decline in weight was uninterrupted throughout the experimental period. Each rat of the second group was given in addition to the basal ration the total of its own feces for the first week, and for the remainder of the experimental period the daily feces supplement was reduced to 1.0 gm. per day if the amounts excreted exceeded this quantity and the entire amounts excreted if these were less than 1.0 gm. per day. The feces consumed by these animals ranged from 66 to 97% of the amounts excreted after the first week.

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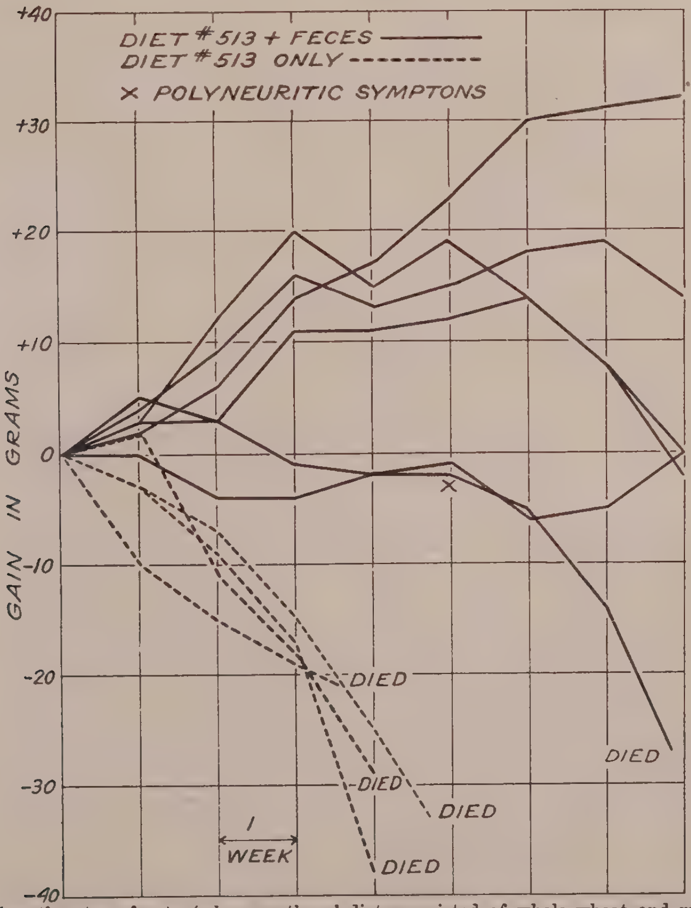
[7] Steenbock, H., Sell, M. T., and Nelson, E. M., *J. Biol. Chem.*, 1923, **55**, 399.

[8] Sherman, H. C., and Chase, E. F., *J. Am. Chem. Soc.*, 1931, **53**, 3506.

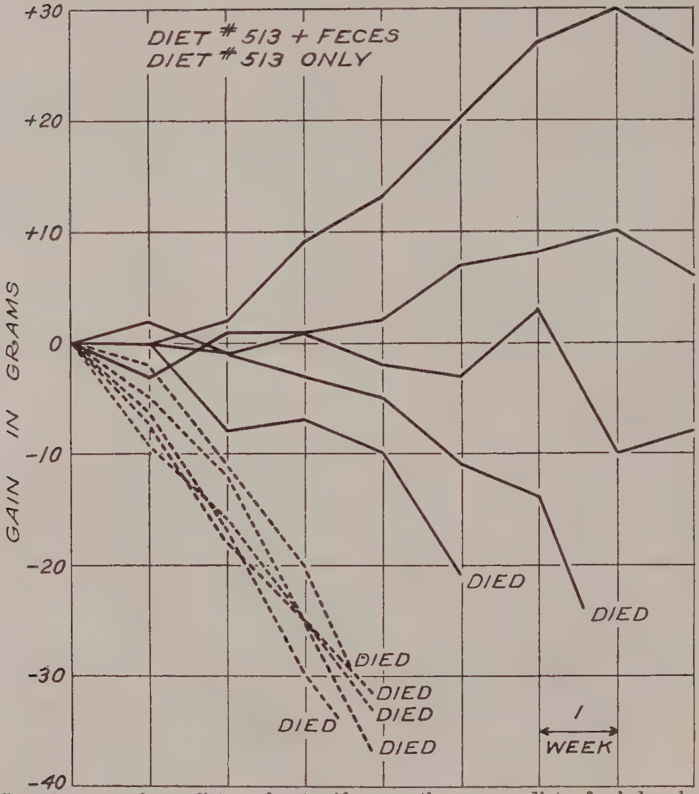
All of the rats receiving their own feces as a supplement to the vitamin B(B_1) deficient ration were distinctly benefited by this supplement as shown by the fact that they invariably lived longer and either lost weight less rapidly than the control animals or grew, usually at a very slow rate. Of the 11 animals which were fed feces only one developed symptoms of polyneuritis, 3 died before the end of the 8 weeks experimental period having shown a slow decline from the start, and 8 grew but did not grow continuously for 8

FIG. I



Growth rates of rats (whose mothers' diets consisted of whole wheat and milk powder) on a vitamin B(B_1) deficient diet with and without addenda of their own feces.

FIG. II



Growth rates of *one* litter of rats (from mothers on a diet of whole wheat, whole milk powder and lean beef) on a vitamin $B(B_1)$ deficient diet with and without addenda of their own feces.

weeks, indicating that the feces had become less potent as vitamin $B(B_1)$ sources or that a second growth limiting factor had become exhausted or insufficient. The growth curves of the rats taken from two previous nutritional histories are shown in figures 1 and 2. Three animals not included in the figures made small gains in body weight when fed 100% of their own feces for one week, but lost weight promptly and steadily when the feces were omitted from their ration in spite of the occasional acquisition of feces due to artifices on the part of the animals.

Conclusions. (1) Eleven rats receiving the Sherman and Chase basal ration deficient in vitamin $B(B_1)$ and containing 53% of *raw* cornstarch, when fed 66 to 97% of their own feces as addenda were benefited as compared with littermate controls on the same ration without addenda. (2) The greatest growth resulting from feeding the rats 66 to 97% of their own feces was 4 gm. per week; most of the animals grew much less or declined steadily but more slowly than the control animals. (3) It would seem that vitamin $B(B_1)$ studies, using the assay method outlined by Sherman and Chase, were not seriously encumbered with disturbing influences of coprophagy since the small amounts of feces ordinarily consumed by the animals are not sufficient to influence their growth rates appreciably.

6365

Bacteriophage in Experimental Staphylococcal Septicemia.

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The principles of phage therapy have been developed upon 2 cardinal assumptions: 1. That an active homologous bacteriophage introduced into a host harboring a pathogenic organism in the tissues will propagate at the expense of the disease incitant, eventually causing the latter's widespread lytic destruction and constituting as a consequence a benign *therapia sterilisans* with no danger of untoward response on the host's part such as might attend use of germicidal drugs. 2. That phage lysates contain in solution certain constituents of bacterial cells released at the time of lysis. These substances are capable of inducing antibody responses upon contact with animal tissues and may be expected effectively to produce an active immunity. With a few notable exceptions, studies on phage therapy in experimental infections have been directed largely toward evaluation of phage as a possible therapeutic modality, as is, of course, also the case with purely clinical observations. However, the primary action anticipated in the application of phage to the treatment of diseases, i. e., massive bacterial dissolution *in vivo* has not been subjected to any critical experimental analysis so far as the authors are aware. Since any accepted concept of phage action will have definite influence upon the manner in which phage is applied clinically and also upon the nature and extent of our clinical expectations, a series of experiments was undertaken to investigate whether conditions essential for bacterial lysis can develop in animal tissues and if so just how much lytic destruction of susceptible organisms is produced. Previous work by Krueger and Northrop[1, 2, 3] on the reaction occurring between a staphylococcus and antistaphylococcus phage showed that under controlled *in vitro* conditions bacterial growth is an essential conditioning factor for phage formation and that the percentage rate of increase of phage is proportional to the percentage rate of increase of bacteria, i. e., $\frac{dP}{dt} \propto C \times \frac{dB}{dt}$. Phage accumulates within the bacteria meanwhile maintaining equilibrium with phage in the surrounding medium until a certain critical concentration of phage per bacterium is attained, when lysis ensues. There is thus a definite lytic threshold. Further, phage is distributed between susceptible cells and the medium in 2 ways; with live cells, either resting or growing, distribution is of normal type and diffusion of phage into or out of the organisms proceeds in the direction of maintaining equilibrium. With dead cells, however, phage is adsorbed irreversibly and equilibrium may be represented in terms of Freundlich's adsorption isotherm equation.

The infection chosen for the present study, an acute septicemia produced by intravenous injection of the identical strain of staphylococcus into rabbits, was selected rather than a chronic pyemic state in order to limit phage effects to actual lysis of organisms and to obviate as far as possible the antibody responses which may occur in more prolonged infections. There were available accurate methods for quantitative determination of the phage[4] and of staphylococci.[5] For each experiment 3 rabbits received intravenously a certain number of washed staphylococci per kilo of body weight. One animal was immediately given an intravenous dose of standard bacteriophage calculated on a kilogram basis as adequate to furnish a titrable concentration of phage in the blood stream. A second animal received a like amount of broth and the third animal was given nothing at all.

Blood samples were taken at intervals for the purpose of determining the concentration of bacteria and the concentration of phage per ml. of circulating blood. In no instance was it possible to detect any increase whatsoever in phage/ml of blood and there never devel-

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[1] Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1930, **14**, 223.

[2] Northrop, J. H., and Krueger, A. P., *J. Gen. Physiol.*, 1931, **15**, 329.

[3] Krueger, A. P., *J. Gen. Physiol.*, 1930, **14**, 493.

[4] Krueger, A. P., *J. Gen. Physiol.*, 1929, **13**, 557.

[5] Krueger, A. P., *J. Gen. Physiol.*, 1929, **13**, 553.

oped in the blood stream a ratio of phage to bacteria adequate for initiation of lysis even when the amount of phage injected was greatly in excess of the amount required to lyse the number of bacteria used. The bacterial curves in treated animals and controls exhibited no significant differences. Bacteria recovered from the blood stream both in fatal infections and in animals who survived (because they received less than the lethal dose of organisms) showed no change as regards susceptibility to phage action. That is, exposure to phage in the circulating blood did not produce phage resistant strains.

Red cells were found to be capable of adsorbing phage in quantities sufficient to make this action a considerable factor in the failure to establish a lytic threshold *in vivo*.

Our experiments indicate that: 1. Homologous anti-staphylococcus bacteriophage introduced in large quantities into the blood stream of rabbits suffering from acute experimental staphylococcal septicemia not only does not increase in amount but is rapidly eliminated from the blood stream. 2. The phage has no influence on the course of the experimental infection nor upon the quantities of bacteria found in the blood stream. 3. In the case of the infection studied it is not possible to establish in the circulating blood those conditions shown in earlier work to be requisite for bacterial lysis. 4. Phage adsorption on red blood cells effectively operates to remove considerable quantities of phage from participation in lysis. 5. Clinical failures in the treatment of staphylococcal septicemia with bacteriophage, such therapy aiming at lytic dissolution of cocci in the blood stream, are not only explicable but are to be anticipated in light of known facts concerning the bacterium-bacteriophage reaction.

6366

Meningococcus Precipitinogens in the Cerebrospinal Fluid.

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The demonstration of type-specific substances for Group I-III and Type II strains of meningococcus,[1] and the production of sera rich in precipitins for such substances but lacking in precipitins for the non-type-specific (C and P) fractions have led to the investigation of C.S.F. (cerebrospinal fluid) from meningococcal meningitis to ascertain whether this contains type-specific precipitinogens. Vincent and Bellot[2, 3] and others have demonstrated precipitin reactions between C.S.F. and polyvalent antimeningococcal serum. Most of the investigators noted some inconsistencies, especially cross-precipitation with C.S.F. of other forms of meningitis, notably pneumococcal. The reason for this, no doubt, lies in the fact that the polyvalent serum contains non-specific antibodies, and that the antibody for the C polysaccharide of the meningococcus will react with the similar fraction from other organisms, especially the pneumococcus. Using polyvalent serum, there is, of course, no possibility of typing the organism involved. Marie,[4] however, states that cases of meningitis due to the meningococcus can be separated from those due to the parameningococcus by the use of monovalent serum prepared against these 2 types and used in the precipitin reaction with C.S.F.

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[1] **Rake, G., Proc. Soc. Exp. Biol. and Med., 1931, 29, 287.**

The spinal fluid is cleared by rapid centrifugation. The precipitin test is made with equal parts of undiluted serum and spinal fluid for 2 hours at 37° C. and 15 to 18 hours in the ice-box. Both the ring method and the mixing method have been used. 0.1 cc. of each of the 3 type sera is placed in a small tube. 0.1 cc. of spinal fluid is layered on top of each specimen of serum so that the precipitate appears as a ring at the point of junction. A reading is made immediately and again after one hour at 37° C. The tubes are then agitated and returned to the water bath. At the end of 2 hours a third reading is made and the tubes are placed in the ice-box overnight for a final reading in the morning. Of all the specimens of cerebrospinal fluid tested, only those are mentioned here from

TABLE I.

C. S. F. No.	Type by Agglutination	Type I Serum		Type II Serum		Type III Serum	
		2 hr.	20 hr.	2 hr.	20 hr.	2 hr.	20 hr.
23	I-III	++	++±	0	0	+	++
28	I-III	+	+	0	0	±	+
425	I-III	+	+++	0	0	±	++
429	II	0	0	±	+	0	0
436	II	0	0	±	±	0	0
449	I-III	0	(±)	0	0	(±)	±
451	I-III	+	++	0	0	+	++±

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[2] Vincent, H., and Bellot, *Bull. Acad. Med.*, 1909, **61**, 326.

[3] Vincent, H., and Bellot, *Bull. et Mém. Soc. Hôp. de Paris*, 1909, **27**, 952.

[4] Marie, P. L., *Bull. et Mém. Soc. Hôp. de Paris*, 1917, **41**, 259.

which the meningococcal strain involved was grown and typed in this laboratory. Seven fluids fulfill this requirement and the results of precipitin and agglutinin tests on these are given in Table I. It will be seen that the precipitin results agree completely with those of agglutination and there is no cross-precipitation. The apparent cross-precipitation between Types I and III can be explained by the fact that there is no demonstrable difference in the type-specific substance of these two types as they occur at present, if indeed the types themselves differ. The custom has therefore been adopted of referring to these strains as belonging to Group I-III rather than to either Type I or III.

The presence of the type-specific substance in C.S.F. is probably due to autolysis of the organisms and it has been found that fluids such as No. 436 and No. 449, in which the precipitin reaction is weak, show very few organisms on the smear, whereas in cases as No. 425 and No. 451, in which the reaction is strong, meningococci are present in large numbers.

It is important that C.S.F. be obtained before intrathecal serum treatment or early in the course of such treatment. Fluids positive before such therapy rapidly become negative or very weak in reaction. One must suppose that antibodies in the serum unite with the specific substance and remove it from solution. C.S.F. stored in the ice-box will continue to give a precipitin reaction for many months as will solutions of the isolated type-specific substance.

These observations offer a rapid method of typing a case of meningococcal meningitis and remove one of the chief criticisms against the use of monovalent therapeutic serum, namely, the enforced delay of 2 or more days while the strain is being grown and typed. Readings such as those in Table 1 can be made 17 to 20 hours after the spinal fluid is drawn, but the majority of fluids will allow of a diagnosis on removal from the water bath at the end of 2 hours. In 4 of the fluids shown in Table 1 a diagnosis could have been made within 2½ hours after the fluid was withdrawn. In many cases, indeed, the precipitin reaction is immediate and sufficiently strong to allow of a diagnosis directly fluid and serum come into contact.

6367

Relationship Between Acute Liver Injury Induced by Alcohol, Retention in Plasma of Phenoltetrachlorphthalein and Elimination of Phenolsulphonephthalein.*

WM. DE B. MAC NIDER.

From the Laboratory of Pharmacology, the University of North Carolina.

The observation was made that dogs acutely intoxicated by ethyl alcohol in a certain number of experiments showed an increase over the normal in the elimination of phenolsulphonephthalein by the kidneys.[1] Such an increase was transitory, developing within 3 hours after the use of the alcohol and decreasing if the alcohol was withheld from 48 to 72 hours. The increase in the output of phenolsulphonephthalein by this group of dogs was associated with an edema and fatty infiltration of the liver lobules which commenced at their periphery and involved from one-half to two-thirds of their substance. The conclusion was made that the increase in the elimination of this dye was due to the liver failing to destroy a portion of it which would increase the amount available for elimination by the kidney. Similar observations have been made by Hanner and Whipple[2] on the relationship between a liver necrosis induced by chloroform and an increase in the elimination of this dye.

In the present preliminary study 12 dogs have been employed to ascertain the relationship, if any, during an acute liver injury from alcohol between the percentage retention and rate of disappearance from the plasma of phenoltetrachlorphthalein and the percentage elimination of phenolsulphonephthalein by the kidneys. Four of the animals were not given alcohol. These serve as controls. The dogs were starved for 2 days. No restriction was made on the water intake. The control dogs were then lightly anesthetized by a morphine-ether anesthesia and liver tissue removed for histological study. The remaining 8 animals were given 10 to 15 cc. of a 40% solution of ethyl alcohol by stomach tube and kept in a state of semialcoholic anesthesia by repeating the alcohol for 12 hours. At the end of such periods they were also lightly anesthetized with a similar anesthetic and liver tissue obtained for study.

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* This investigation was made possible by the Edward N. Gibbs Prize Fund of The New York Academy of Medicine.

[1] MacNider, Wm. deB., and Donnelly, G. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 586.

[2] Hanner, J. P., and Whipple, G. H., *Arch. Int. Med.*, 1931, **48**, 598.

The use of phenoltetrachlorphthalein as a test for liver function was conducted according to the technique devised by Rosenthal,[3] while the phenolsulphonephthalein elimination was estimated according to the method of Rountree and Geraghty.[4] The phenoltetrachlorphthalein estimations were made immediately following the short period of anesthesia and also at the expiration of one hour following the introduction of the dye. The phenolsulphonephthalein determinations were made 2 hours later and represent the total output of this dye in a 2 hour period. The course of the following experiments, 2 from the control group and 2 from the animals intoxicated by alcohol, are characteristic for the 2 groups.

Control Animals. The dog of Exp. 1 showed a concentration of phenoltetrachlorphthalein in the plasma of 8%. At the end of an hour the plasma was negative for the dye. The elimination of phenolsulphonephthalein by the kidneys was 66%. The control animal of Exp. 4 had a plasma concentration of phenoltetrachlorphthalein of only 6.6% with a disappearance of the dye from the plasma within an hour. The elimination of phenolsulphonephthalein was 54%.

The livers of these animals were normal. There was no edema or necrosis of the liver cells. The nuclei stained normally. Fine dust like particles of lipoid material could be detected in the cells when such tissue was fixed in formaline and stained with Scharlach R. by Hersheimer's method.

Animals Intoxicated with Alcohol. The dog of Exp. 3, after an alcoholic intoxication lasting for 12 hours showed a phenoltetrachlorphthalein concentration in the plasma of 16% and a retention of the dye of 7% at the end of one hour. The elimination of phenolsulphonephthalein by this animal was 83%. The dog of Exp. 11 gave a plasma concentration of 27.8% with a retention at the end of an hour of 17.3%. In this animal there occurred an elimination of phenolsulphonephthalein in a 2 hour period of 93%.

The livers of the dogs intoxicated by alcohol have shown a reaction similar to that previously[1] described. A marked edema usually without necrosis of the cells, a failure of the nuclei to stain normally and the presence of variable amounts of stainable lipoid material. These changes commence and are most marked in the periphery of the lubules. The extent of the injury has varied from one-half to over two-thirds of a liver lobule. In those animals with the most marked evidence of such an injury the initial concentration of phenoltetrachlorphthalein in the plasma has been higher, its presence has persisted for an hour and the elimination of phenolsulphonephthalein has been greater than in the animals with a less marked liver injury.

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[3] Rosenthal, S. M., *J. Am. Med. Assn.*, 1922, **79**, 2151.

[4] Rountree, L. G., and Geraghty, J. T., *J. Pharm. and Exp. Therap.*, 1909, **1**, 579.

The use of phenoltetrachlorphthalein as a test for liver function indicates a definite relationship between the degree of liver injury and the percentage elimination of phenolsulphonephthalein by the kidneys.

6368

Further Studies on the Life Cycle of the Avian Tubercle Bacillus.

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From the Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh.

The primary object of this paper is to indicate briefly that the experimental high lights of a life cycle for the tubercle bacillus have been consummated. The cycle at present consists of 4 stabilized stages which are vegetative, and 3 transition stages between them which are reproductive or gonidial—7 in all. Of the reproductive stages 2 are probably asexual, and inquiry into the *possible* sexual nature of the other will be reported in a subsequent paper.

Each important stage has been thoroughly single-celled before progression to the next stage has been induced by germination of its appropriate gonidial form; which form constitutes the cytologic mechanism for the transition. For this reason Stages 2, 4, and 6 should perhaps be designated as "phases"* except for possible confusion in the text. Stage 1 is the S form of the avian tubercle bacillus which gives rise to Stage 2, which is filterable.[1, 2, 3] It in turn germinates as Stage 3 in stabilized diplococcus form. As a transition form of Stage 3 is the large tetrad form, Stage 4, which gives rise to the stabilized diphtheroid Stage 5, which in turn yields acid-fast gonidia constituting Stage 6. These latter then germinate as the R form of the tubercle bacillus—Stage 7—and then presumably to the original S form. The cycle's completion rests only on transformation of the R to the S form, already well demonstrated for the avian bacillus by others. Stages 1, 6, and 7 are acid-fast; Stages 2, 3, 4, and 5 are non-acid-fast.

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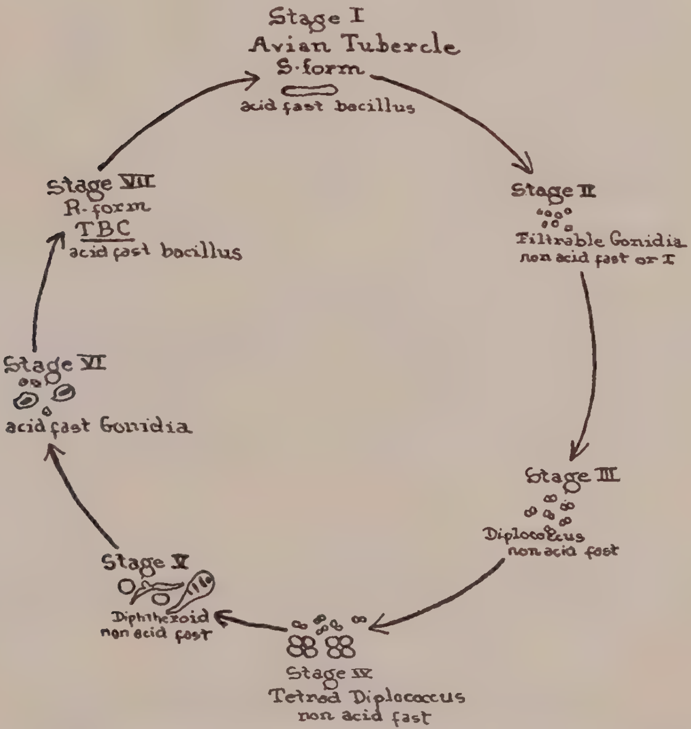
* The term "phase" indicates an absence of detectable multiplication of such a form before its germination into a "stage", which is capable of vegetative multiplication for many "generations".

[1] Mellon, Ralph R., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 206.

[2] Mellon, Ralph R., and Fisher, L. W., *J. Bact.*, 1932, **23**, 18.

[3] Mellon, Ralph R., and Fisher, L. W., *J. Inf. Dis.*, 1932, **51**, 117.

Diagram of the Life Cycle
of the Tubercle Bacillus



It is noteworthy that the stabilized diphtheroid Stage 5 was transformed to the R form of the tubercle bacillus (Stage 7) on 6 occasions by reason of breaking the dormancy of the acid-fast gonidia, by special methods to be detailed later. Most of the observations indicate its origin from the acid-fast spore-like gonidia, although it is not certain that it is limited to these. It appears that these coccoidal structures throw out a germ tube, as does a fungal spore, which elongates into an acid-fast filament, which simultaneously fragments to ordinary tubercle bacilli.

We have previously shown[1, 2, 3] that the non-acid-fast filtrable phase of a saprophytic acid-fast bacillus may revert to the original directly, or by way of the diphtheroid without its stabilization. But the interpolation of several stabilized non-acid-fast cyclostages between the filtrable form and the original is now shown for the tubercle bacillus. This establishes conclusively the reproductive or gonidial status of the filtrable form, which cannot be of "involution" nature.

Summary. Dissociation of the tubercle bacillus into a heterogeneous non-acid-fast progeny and its occasional reversion to the original is a very old observation. The S and R variability reversions are more recent, while demonstration of a filtrable phase (granules of Much and their congeners) for pure cultures is quite recent. The sum total of these observations constitutes a collection of "variability fragments", so to speak; but it requires orderly allocation by experiment to dispel the chaos that they have represented to monomorphic theory.

These studies represent, I believe, the first experimental integration of these several "variability fragments" in accordance with a single unifying conception, namely, the biologic sequences of a true life cycle. Single-celled cultures only have been employed. The variability of the non-acid-fast progeny is of such a character as to suggest strongly its implication with the genetic mechanism of the cell, which accounts in part for the following paper.[4]

6369

Therapeutic Effect of Specific Immune Serums Against a Metazoan Parasite (Cysticercus Fasciolaris)*

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From the Department of Zoology, Washington University, St. Louis.

Since it had been found possible[1] to protect the rat against infection with *Cysticercus fasciolaris* by means of serums from rats infected with this cestode or artificially immunized by powdered worm, experiments were then carried out to determine whether such serums, given after infection, would inhibit development of the cysticerci. Accordingly rats were fed, by stomach tube, equal portions of a uniform suspension of onchospheres, and pooled serum from immune rats (infected with *C. fasciolaris*) was injected intraperitoneally, in proportion to body weight, at varying intervals after infection. Control animals of one group received normal rat serum, and those of another group were not given serum.

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[4] Lindegren, Carl C., and Mellon, Ralph R., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 110.

* This investigation was made possible by a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Miller, H. M., Jr., and Gardiner, M. L., *Science*, 1932, **75**, 270; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 779; *J. Prev. Med.*, in press.

The results of 3 experiments with more than 140 animals show that the immune serums were effective in arresting the development of *C. fasciolaris,* when administered within about 9 days after the rats were fed onchospheres. At autopsy 28 days later cysts were completely, or almost completely absent in the animals of these groups, while numbers of large cysts (3 to 6 mm. diameter) were present in the livers of the rats of control groups. Cyst development was but slightly hindered in the animals of groups to which immune serum was given later than 9 days after infection.

The results of one experiment are given in Table I.

TABLE I.
Figures give the average number of cysts in liver.

Group	Injected with†	Days after Infection	No. of Rats	Cysts Living	Cysts Dead
Control			7	52	43
"	Normal rat serum	4	7	112	38
A.	Serum from infected rats	4	7	0.3	17
B.	" " " "	5	7	2	27
C.	" " " "	6	8	1	44
D.	" " " "	7	7	9‡	80
E.	" " " "	8	7	0.7	81
F.	" " " "	10	7	60	75

† 1 cc. of serum per 100 gm. body weight.
‡ 1 rat with 57 cysts raised this average from a fraction to 8.7.

6370

Effect of Certain Extracts on Basal Metabolism of Guinea Pigs.

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Urine from patients with toxic goiter was obtained in 5 gallon lots from such patients, concentrated and extracted with petroleum ether as well as alcohol. An equal amount of urine from presumably normal people was obtained, concentrated and extracted in the same manner, to be used as control extracts. Equal amounts of the extract from the urine of patients with toxic goiter and the urine of normal people were fed to guinea pigs of the same weight, which had had a sufficient number of metabolic readings to determine a normal base line. The animals were starved 16 to 22 hours before the metabolic determination which was usually done every second day.

The apparatus used was a closed chamber system, utilizing the Benedict principle of measuring oxygen consumption with a spirometer without regard to the output of carbon dioxide, which was taken up by passage of the gaseous mixture through soda lime.

Various extracts of concentrated urine from normal adults and from patients with toxic goiter were made. The only ones which seemed potent from the standpoint of influence on basal metabolism were the extracts made with petroleum ether. However, the presence of so many toxic elements in our alcoholic extracts of urine does not allow us to form any conclusions regarding this particular extract. It was found that the animals to which the extract of urine from patients with toxic goiter was given sustained a slight increase in basal metabolism. The highest level was an average of plus 18% with observations over a period of 3 weeks. Each of another series of 3 animals was fed 8 mg. desiccated thyroid per kilo per day. One was fed over a period of 3 weeks with a petroleum ether extract of urine from supposedly normal adults, another fed in a similar manner with a petroleum ether extract of urine from a patient with toxic goiter, whereas the third animal was given nothing except the desiccated thyroid. Each animal was fed the same amount of a standard balanced diet, the same periods of starvation preceding each basal determination. The animal which was fed thyroid alone, had an average basal metabolic reading of +15% above its preliminary base line, the animal which was fed thyroid and extract of urine from a patient with toxic goiter had an average of +26%, whereas the third animal which was fed thyroid and extract of normal urine had an average metabolic reading of —1. The weight loss was 6%, 12%, and 9% respectively. It appeared, therefore, that the extract of urine from the patient with toxic goiter increased the toxicity of the desiccated thyroid, whereas the extract of urine from normal people had tended to counteract the effect of the desiccated thyroid.

While studying the effect of variation in diet upon the basal metabolism of guinea pigs, it was discovered that a diet consisting chiefly of lettuce and cabbage, especially the former, caused a decrease in the metabolic level in spite of the fact that there was a drop in weight while the animals were fed lettuce or cabbage alone.

Since lettuce or cabbage alone constitutes an unbalanced diet, we made various extracts of lettuce (which seemed to produce a greater depression than cabbage) with ether, alcohol and water and fed each to guinea pigs. When animals were then fed on a balanced diet it was found that an alcoholic or aqueous extract of lettuce would cause a depression of the animal's basal metabolism. The ether extract did not appear to be so effective. Each animal was fed extract from an amount of lettuce equal to about ½ to 1 pound of the green vegetable per kilo of body weight per day. On several occasions the average metabolic curve under such feedings averaged between —10% and —20% below the preliminary base line. Most of these animals showed a slight gain in weight during the observations.

When desiccated thyroid is fed along with the lettuce extract there appears to be a definite tendency for the extract to diminish the effect of the thyroid, but in no instance did the extract completely counteract the effect of the thyroid. However, it appears to be more difficult to protect the animal against thyroxin by this means.

6371

Experimental Trichiniasis Infections in Monkeys.*

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Apparently no experimental infections with *Trichinella spiralis* have been attempted in monkeys. In a summer's program of work at the Gorgas Memorial Laboratory, Panama, the following 7 species of Central American monkeys were found susceptible to infection with this parasite: the white-throated monkey, *Cebus capucinus,* the black spider, *Ateles dariensis,* the red spider, *Ateles geoffroyi,* the brown and black howler, *Alouatta palliata palliata* and *A. palliata inconsonans,* the marmoset, *Leontocebus geoffroyi,* the yellow titi, *Saimiri örstedii,* and the night monkey, *Aotus zonalis.*

All infections were given according to the body weight of the

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* This investigation was aided by a grant from the Rockefeller Foundation.

animals with counted numbers of trichina larvae obtained free from infected muscle by artificial digestion, and administered through a stomach tube. Doses of 5 larvae or more per gm. of body weight were invariably fatal, death occurring in from 3 to 41 days, depending on the size of the dose (Table I). Symptoms of fever, edema, and a marked eosinophilia, which characterize trichiniasis in human beings, did not develop in the monkeys. It will be noted (Table I) that considerable numbers of adult worms were still present in the intestines of monkeys that died 4 to 6 weeks after infection.

TABLE I.
Course of Infections with *Trichinella spiralis* in the White-Throated Monkey, *Cebus capucinus.*

Monkey No.	Larvae per gm. body wt.	No. larvae fed	Result	No. adults in intestine	% of larvae fed
119	40	59,000	Died in 3 days	—	—
123	20	31,600	" " 14 "	14,000	44
120	10	11,600	" " 6 "	—	—
192	10	8,000	" " 25 "	4,900	61
207	7	8,000	" " 26 "	7,300	91
122	5	10,100	" " 20 "	7,700	77
117	5	8,000	" " 32 "	3,100	38
118	5	5,600	" " 41 "	2,500	45
124	2	4,000	Survived	—	—
121	2	1,900	"	—	—
116	2	2,100	"	—	—

The number of larvae that developed in the muscles in 6 monkeys was determined by grinding up and digesting the whole carcass. The relation of the number of larvae in the muscle to the number of larvae fed to the these monkeys ranged from 396 times to 1,108 times the infecting dose and averaged 698 times. These figures indicate that each female trichina produced about 1,500 larvae during the course of the infection.

Other experiments[1] have shown that rats develop a very definite immunity to reinfection with *Trichinella spiralis*. Attempts to demonstrate a similar immunity in 12 monkeys that had recovered from trichiniasis were not uniformly successful. However, in 5 monkeys including 2 that had received repeated infections, there was evidence that a partial immunity had developed. A more complete report of these experiments will be made later.

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[1] McCoy, O. R., *Am. J. Hyg.*, 1931, **14**, 484.

6372

An Improvement in Experimental Method for Investigation of Vitamin G.

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Several investigators have reported that coprophagy may be a disturbing factor in vitamin B studies. In our own laboratory we have not encountered any serious difficulty with coprophagy in our vitamin B (B_1) studies when the experimental animals were kept on raised wide-mesh screens. On the other hand we have of late observed the practice of coprophagy by animals on vitamin G deficient diets. The feces of these rats are apparently very potent sources of vitamin G, for even the small proportion of the feces which the artifices of the animals enable them to acquire in spite of large meshed raised bottom cages is sufficient to affect the accuracy and precision of vitamin G measurements.

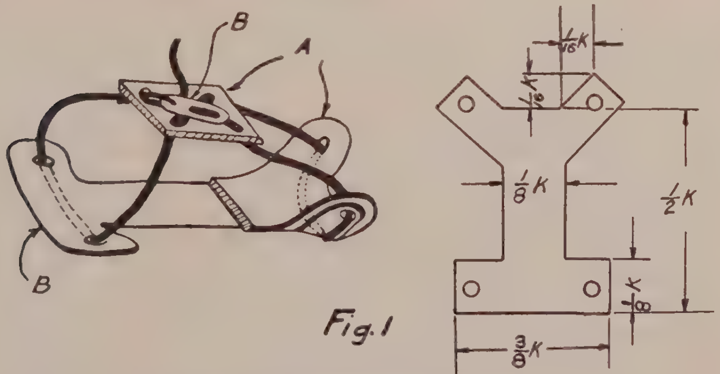

FIG. 1. Anti-coprophagy Harness.
K = size of animal, measured from neck to base of tail.
A = parts made of soft, thin kid.
B = parts made of aluminum (0.009 in. thick).
Note—an allowance for rolled edges of 1/16″-1/8″ should be made.
—Leather fastened to aluminum with Duco cement.

Figure I is a diagram of a small aluminum and leather harness adapted to the prevention of the practice of coprophagy by our experimental animals. The use of this harness not only shortens the period necessary for the young 28-day-old rats to reach stationary weight on the vitamin G deficient diet, but having reached stationary weight the harnessed control animals without vitamin G supplements lose weight more rapidly than unharnessed ones. In one experiment 37 twenty-eight-day-old animals were given the Sherman and Bourquin vitamin G deficient diet until they had reached stationary weight. They were then divided into harnessed and unharnessed groups and each rat was given a vitamin G supplement of 2.0 gm. of skimmed milk powder per week for a period of 8 weeks. The average gain of the unharnessed group for the first 4 weeks was 26.9 gm. with an A.D. of ±2.0 gm., while the average gain of the harnessed rats was 15.4 gm. with an A.D. of ±1.3 gm. The average gain of the unharnessed rats at the end of 8 weeks was 38.8 (A.D. ±4.0) gm., and of the harnessed rats 32.1 (A.D. ±2.1) gm. The prevention of the practice of coprophagy not only decreased the growth rate on limited supplements of vitamin G but also decreased the variation in our experimental results.

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Placental Transmission of Alimentary Anaphylaxis.

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The human and guinea pig placentae are permeable to native proteins and to anaphylactic antibodies, therefore, sensitization may be actively or passively induced in the fetus.[1] It has further been demonstrated that the female guinea pig may sensitize its offspring *in utero* by the inhalation of dry antigenic dusts during pregnancy.[2] Rosenau and Anderson[3] first showed that guinea pigs can be sensitized through feeding. In the experiments detailed below it is shown that a pregnant guinea pig fed an antigenic substance can become sensitized and, in addition, actively sensitize her fetus *in utero*. Pregnant women who are allergic can passively sensitize their offspring to the same substance to which they are sensitive.[4] Normal pregnant women who eat excessively of protein foods may actively sensitize the fetus *in utero,* and when such offspring partake of these particular foods for the first time may manifest allergic reactions.[5]

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[1] Ratner, B., Jackson, H. C., and Gruehl, H. L., *J. Immunol.,* 1927, **14**, 249.

[2] Ratner, B., and Gruehl, H. L., *J. Exp. Med.,* 1929, **49**, 833.

[3] Rosenau, M. J., and Anderson, J. F., *Hyg. Lab. Bull.,* 1906, **29**, 67.

[4] Ratner, B., and Greenburgh, J. E., *J. Allergy,* 1932, **3**, 149.

[5] Ratner, B., *Am. J. Dis. Child.,* 1928, **36**, 277.

Large normal female guinea pigs were fed horse serum one to several days before parturition. The animals were placed in an empty metal cage without food or water for 3 to 5 hours before the sensitizing feedings were given, in order to partially empty their stomachs. They were fed by pipette. Any liquid which dripped from their mouths was caught in a dish, taken up in the pipette and refed to the animal. so that the total amount ingested was recorded. If the liquid was given too fast, the animals would choke and cough, so that extreme caution was observed in emptying the pipette very slowly. If an animal did cough, this was carefully noted, in order that false results due to possible passage of the antigen into the respiratory tract would be avoided. The animals took 25 cc. readily at one feeding and where larger amounts were given, 25 cc. feedings at least an hour apart were administered.

Pregnant animals must be handled with extreme care in order not to precipitate a miscarriage. In spite of such precaution, these experiments were difficult to consummate for often the mother animals died during or soon after parturition, or the offspring did not live long enough to be tested. We succeeded in carrying out tests on 23 families. An additional difficulty was the timing, so that the feeding might be given as near parturition as possible, which is necessary to meet the criteria we have laid down[1] for active sensitization of a fetus *in utero*.

At varying intervals after birth, the offspring were given their primary injection of horse serum. In 8 of the families one or more of the offspring, or 25%, were shown to be sensitive as a result of the antepartum feedings of the mother. The mothers in turn were given their primary injection of horse serum, usually on the same day on which the offspring were tested, and 70% of these were shown to have become sensitized, Table I.

In 3 families (Nos. 1, 12, 16) the offspring were sensitized to equal degrees. The 3 offspring of family No. 16 died in anaphylaxis from their first injection of horse serum. In family No. 1 the offspring were born the day following the feeding of the mother, therefore, the sensitization of the offspring demonstrated 48 days after birth must have been active, due to the passage of the antigen via the placenta into the fetal circulation.

Three other litters (Nos. 6, 7, 8) also fall into the category of active sensitization *in utero,* since the interval of time between the mother's feeding and the birth of the offspring was only 4 days in 2 instances, and 5 days in the other. In family No. 8 the one offspring, injected one day postpartum, was negative, showing that

TABLE I.

Placental Transmission of Alimentary Anaphylaxis.

No.	Am't fed pregnant animal cc.	Days Ante-partum	Offspring			Mother	
			Age Days	No.	Reaction after 1st injection	Days post-partum	Reaction after 1st injection
1	100	1	48	1	+	48	+
			"	2	+		
2	100	1	38	1	0	38	++
			"	2	0		
3	130	1	43	1	0	43	+
			"	2	0		
4	200	2	26	1	0	28	++
			"	2	0		
			"	3	0		
5	100	2	60	1	0	60	+
			"	2	0		
			"	3	0		
			"	4	0		
6	175	4	50	1	0	50	+
			"	2	0		
			"	3	+		
7	200	4	42	1	++	42	+
			"	2	+		
8	160	5	1	1	0	25	+
			25	2	+		
			"	3	+		
9	200	8	28	1	0	28	+
			"	2	+		
			"	3	++		
10	280	8	6	1	0	41	+
			41	2	0		
11	200	9	21	1	+	46	+
			46	2	0		
			"	3	0		
			"	4	0		
12	200	13	22	1	+	22	++++
			"	2	+		
			"	3	+		
13	440	14	2	1	0	35	+++
			35	2	0		
14	520	16	13	1	0	33	+
			33	2	0		
15	580	17	13	1	0	13	+
			"	2	0		
16	460	19	6	1	++++	6	++
			"	2	++++		
			"	3	++++		
17* to 23	80 to 200	1 to 9	4 to 60	21	0	28 to 60	0

0, no anaphylactic reaction; +, dyspnea and scratching; ++, marked dyspnea, convulsive movements; +++, severe dyspnea, convulsions, collapse, final recovery; ++++, typical anaphylactic death with completely ballooned lungs, occurring within a few minutes after injection.

* 7 families, with a total of 21 offspring, were entirely negative. 25% of the total offspring were sensitized as compared with 70% of the mothers, and 38% of the offspring of the positive mothers were sensitized.

no antibodies were transmitted, while the other 2 animals of the same litter were positive at 25 days—a long enough period for the transmitted antigen to cause the production of sensitizing antibodies in the young animals.

In family No. 9 the 3 offspring gave varying reactions. These animals, born 8 days after the mother's sensitizing feedings, received a combination of antigen and antibodies which may account for the varying reactions. In family No. 11 one offspring was positive at 21 days while the other 3 were negative at 46 days. These animals may have received a small amount of early formed antibodies which disappeared before the 46-day period.

It is interesting that in some instances the offspring gave greater reactions than the mother, and in others the reverse was true.

When transmission of sensitization was first described by Rosenau and Anderson,[6] it was the result of transmission of antibodies from mothers to offspring and corroborative evidence for this has been given by various investigators. Wells[7] first showed that guinea pigs could be passively sensitized *in utero* to the natural food ingested by the pregnant female. With this in mind, our original work was started to study the mechanism of sensitization *in utero*. We believe that the whole train of evidence presented by us in previous studies shows clearly that not only can fetuses be sensitized passively, but that active sensitization may also occur. Further, this active sensitization *in utero* can be induced by parenteral injection, by inhalation, and finally, as demonstrated by the above data, by alimentation.

We believe that "congenital protein hypersensitiveness" is thus clearly established, both in the human being and lower animal possessing a permeable placenta, and is significant to the experimenter and to the clinician in all studies on hypersensitiveness, infection, or immunity in the young.

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[6] Rosenau, M. J., and Anderson, J. F., *Hyg. Lab. Bull.*, 1906, **29**, 73.

[7] Wells, H. Gideon, *J. Infect. Dis.*, 1911, **9**, 147.

6374

Procedures in the Isolation and Cultivation of an Organism from Berkefeld N Filtrates of Poliomyelitis Virus.

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I. *Culture Mediums.* A. *"E B" Medium* (Sheep brain after extraction with ethyl alcohol and benzol). Sheep brain, removed aseptically at the slaughter house, was washed thoroughly in sterile physiological salt solution, freed from blood and membranes, and ground finely in a meat chopper. The material was extracted once with 2 volumes of 95% ethyl alcohol overnight (12-16 hours) at room temperature (20-22°C.) and dried in air. The tissue residue was extracted in the same manner twice with 2 volumes of U.S.P. benzol. The resulting finely powdered dry material, in the proportion of 2 gm. to 10 cc. of 0.85% sodium chloride solution, was mixed thoroughly and sterilized in the autoclave at 15 pounds pressure for 20 minutes. The final pH was adjusted to 7.6.

B. *"V B" Medium* (Minced sheep brain in veal infusion broth). This substrate consisted of sterilized minced sheep brain contained in a veal infusion broth to which no peptone had been added.

Fresh sheep brain, removed aseptically at the slaughter house, was washed thoroughly several times in sterile physiological salt solution, freed from blood and membranes, and one kilo of tissue added to one liter of sterile veal infusion broth, pH 8.0, to which no peptone had been added. The mixture was coagulated thoroughly by boiling for 2-3 minutes and 0.2% glucose by volume was added. The cooled material was next passed 2 to 3 times through a meat chopper and minced. Test tubes measuring 1.5x15 cm. were filled to a depth of approximately 6 cm. (roughly 10 cc. by volume) and sterilized in the autoclave at 15 pounds pressure for 20 minutes. A second sterilization at 15 pounds pressure for 10 minutes was given on the following day, whenever the technical procedures in the preparation of the material warranted such added precaution. Frequently the culture medium was sterilized in small flasks and stored. Tubed medium prepared from these supplies was always subjected to a second sterilization at 15 pounds pressure for 10 minutes. The final pH of the medium was adjusted whenever necessary to 7.6. Owing to its natural buffering quality, the culture medium stabilized itself during prolonged incubation at a suitable range of pH varying between 6.6 and 7.0, in the course of cultivation experiments.

II. *Tests for Sterility of Culture Medium.* The degree of heat penetration necessary for proper sterilization and ruling out the possibility of living tissue in the culture medium was determined as follows: A maximum recording thermometer was placed in the center of the material contained in a test tube and held firmly in position 2 cm. from the base. The pressure within the autoclave was raised to 15 pounds and within 5 minutes the temperature registered 118°C. After an exposure of 3 minutes to this temperature, the steam was shut off and the autoclave opened 2 minutes later. At this time the temperature recorded within the tube of medium was 96°C. The tube was allowed to cool spontaneously, and 35 minutes after the beginning of the experiment, the temperature within the tissue mass registered 68°C.

An exposure of 15 to 20 minutes at 15 pounds pressure would therefore be adequate to destroy any living tissue and to effect complete sterilization. The resistance of nervous tissue to high temperatures has been found to be considerably less than that of other tissues. Pincus and Fischer showed that an exposure to 60 or 65°C. for a few minutes was lethal.

Tests for sterility were made by incubating the culture medium at room temperature (20-24°C.) and at 37.5-38°C. for 3 to 4 weeks. As an added precaution 3-5 cc. samples of the medium were transplanted to simple and enriched culture mediums which were carefully observed for 3 to 4 weeks to rule out all possible sources of contamination.

III. *Cultivation of the Virus.* The inoculum was an active poliomyelitis virus that had been used successfully in the production of experimental poliomyelitis. *Macacus rhesus* monkeys were inoculated intracerebrally, under full ether anesthesia, with 0.2 to 0.5 cc. of a 5% saline Berkefeld N filtrate prepared from the brain and cord of a previously infected animal. After definite paralysis had occurred following a typical clinical picture of the disease, the animals were etherized and the brain and spinal cord removed with strict sterile precautions, and preserved in 50% glycerol. The glycerolated material used in the primary cultivation experiments had been stored 5 to 6 months in the icechest at a temperature of 4°C. before inoculation of the culture mediums. In the recultivation experiments the materials prepared from the brain and cord of monkeys that had been infected by means of cultures were inoculated into the mediums after shorter periods of glycerolation ranging from 1 to 4 days with a maximum period of 54 days.

For primary cultivation 0.1 to 1.0 cc. of a Berkefeld N filtrate of a 5% saline suspension of the brain and cord virus was used. The first successful culture, however, was obtained with the least amount of the filtrate, and it is reasonable to assume that the larger amounts may yield more frequent positive cultures. In the recultivation experiments with "recaptured" virus cultures, both the filtrates and suspensions were employed separately in amounts of 1 cc.

Essentially the procedure was to inoculate the virus material into the "E B" medium and to make subplants at suitable intervals to the "V B" substrate in which the cultures were maintained by serial dilution. In a number of instances successful growth was obtained by means of the "V B" medium alone. Undoubtedly the 2 mediums exerted different effects upon growth, morphology and virulence. Reversible morphological changes were unusually striking when observed over long periods of time.

A series of 8 to 10 tubes of "E B" medium and "V B" medium in parallel was inoculated with the Berkefeld N filtrate and incubated at 37.5° to 38°C. Two tubes in each set were left unopened during the 3 to 4 weeks' period of observation when samples from the others were carefully withdrawn for subplants every 7 to 10 days by means of a platinum loop or a sterile capillary pipette (approximately 0.1 cc.), and for film preparations. Control tubes included Berkefeld N filtrates and suspensions of poliomyelitic monkey brain and cord inoculated into ordinary simple fluid and solid culture mediums; filtrates and suspensions from normal monkey brains and from herpes, encephalitis and neurovaccinia; and finally uninoculated "E B" and "V B" mediums.

IV. *Subplants and Film Preparations.* Tubes found to contain ordinary bacteria that might be contaminants were discarded. Generally these could be detected after 24-48 hours of incubation. Cultures that gave macroscopic evidence of growth only after 14-21 days were set aside for study and subplanting. At such times a faint turbidity changing to definite clouding of the culture medium was observed in the deeper levels of the supernatant layer of fluid in the tubes containing "E B" medium. This change was not observed regularly in the "V B" substrate. A sterile platinum loop applied to this layer of material brought away a slightly viscous fluid adhering momentarily by a fine thread as the loop was withdrawn.

Film preparations were allowed to dry on slides *without heat* *fixation* and were stained by the methods of Wright, Giemsa, and Gram. The Wright stain was the method of choice because it gave the least distortion of the organism and brought out the polychromatic details of structure. *It is especially noteworthy that the first generation culture tubes containing filtrates from several strains of poliomyelitis virus showed a definite turbidity in the "E B" medium from which numerous film preparations, however, proved to be negative for growth upon careful microscopic examination. Subsequent transplants to the "V B" culture medium or to the "E B" substrate were found invariably to contain living organisms. Such positive secondary cultures were often demonstrable as early as 10 to 14 days before growth became microscopically discernible in the parent culture tubes.*

Subplants from tubes that yielded growth were made every 5 to 10 days. The second and subsequent generations were found to develop satisfactorily within one week. The unopened reserve tubes were examined after growth had occurred in the remaining series and were found to contain the identical organisms.

Transfers from the tubes showing primary growth with the inoculum were made by means of a single 2 mm. platinum loopful of material for the first 3 or 4 subplants. Thereafter a capillary glass pipette was used, containing approximately 0.1 cc. of the culture. This was done in order to effect many millionfold dilutions of the original virus under cultivation. At the same time transfers were also made into ordinary brain-heart (Bacto) infusion broth and to blood-agar plates in order to control possible contamination and the presence of ordinary bacteria.

From subplants the organisms could be grown only under strict anerobiosis on the surface of a solid culture medium prepared by adding 8 parts of "V B" medium to 10 parts of 2% veal infusion agar containing 0.4% NaH_2PO_4, having a pH of 7.6. This medium in Petri plates, or slanted in test tubes, yielded moderate growth after 7 to 10 days, and more abundant growth after 2 or more weeks, provided ample moisture was present. Successful secondary cultivation in this manner was accomplished by the use of the Boez-Olitsky apparatus equipped with a palladinized asbestos catalyzer. Subplants from these cultures failed to develop on aerobic blood agar plates and ordinary agar slants or in brain-heart infusion (Bacto) incubated aerobically and anerobically under sterile liquid albolene. All these mediums remained negative for macroscopic and microscopic growth after one month of observation.

Although successful cultivation of an anerobic organism from poliomyelitis virus was accomplished by means of the "E B" medium in conjunction with the "V B" medium for subplants, further study will be necessary to establish the possible dependence of either one upon the other. For the recultivation of organisms from monkeys that had been successfully inoculated with the culture, the "V B" medium by itself was generally found satisfactory. However, the questions of adaptation of the strain, its multiplication, and maintaining of its virulence and infective power may be modified profoundly by the sequence of events taking place in the different culture mediums over varying periods of time. These matters must be left for future investigation.

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Agglutination of Streptococcus Hemolyticus by Rheumatoid Arthritis Sera: Thermal Characteristics of the Reaction.

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It has been reported[1, 2] that the majority of sera obtained from cases of rheumatoid arthritis agglutinate hemolytic streptococci in high dilutions at 55°C. Weaver[3] reported that scarlatinal streptococci were agglutinated at room temperature and at 37°C. by sera from cases of pneumonia, typhoid fever and erysipelas at about the same dilutions as by scarlet fever sera. Cultures heated to 60°-62°C. for one hour were no longer agglutinated by such sera. Sera similarly treated also lost their capacity to agglutinate. Tillett and Abernethy[4] recently reported that hemolytic streptococci may be agglutinated by sera obtained from various acute bacterial infections and that the agglutinability is destroyed by heating cultures to the thermal death point.

In the study of the rheumatoid arthritis reaction comparative tests were performed at 37°C. and at 55°C., both with living cultures and cultures killed by heating at 55° for 1-2 hours. Similar tests were carried out on sera obtained from (1) a wide variety of other pathological conditions including many acute febrile diseases, (2) normal individuals, (3) rabbits immunized against streptococcus hemolyticus.

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* The Arthritis Clinic of the Presbyterian Hospital is supported by the Faulkner Memorial Fund.

[1] Nicholls, E. E., and Stainsby, W. J., *J. Clin. Invest.*, 1931, **10**, 323.

[2] Dawson, M. H., Olmstead, Miriam, and Boots, R. H., *J. Immunol.*, 1932, **23**, 187, 205.

[3] Weaver, G. H., *J. Infect. Dis.*, 1904, **1**, 91.

[4] Tillett, W. S., and Abernethy, T. J., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 270.

The results of these tests are summarized as follows: Some normal human sera agglutinate living cultures of *Streptococcus hemolyticus* at 37°C. Little, if any, agglutination occurs at 55°C. and the reaction is almost entirely abolished by heating the cultures at 55°C. for one hour. Heating the serum at 55°C. for half an hour diminishes its capacity to agglutinate. Sera from certain acute febrile diseases, *e. g.*, typhoid fever, may agglutinate living cultures in high dilutions at 37°C. but only slightly at 55°C. The reaction is destroyed or greatly diminished by heating the culture at 55°C. for one hour. Agglutination is also greatly diminished by heating the sera at 55°C. for half an hour. The rheumatoid arthritis sera that agglutinate hemolytic streptococci react in a different manner. Such sera agglutinate equally well at 37°C. and at 55°C. Heating either the serum or the culture at 55° has little, if any, effect on the reaction. Immune rabbit sera react in the same manner as rheumatoid arthritis sera except that the agglutinins withstand a slightly higher degree of heat.

Similar tests have been carried out with sera from cases of subacute bacterial endocarditis and strains of streptococci isolated from the blood-stream of such cases. The tests indicate that these sera react in the same manner as rheumatoid arthritis sera in respect to the thermostability of agglutinins and agglutinogens.

Considerable difficulty has been encountered in obtaining uniform and consistent results with cultures grown in various lots of plain broth. Certain lots of broth were found to yield growths which were unsuitable for differential tests.

Conclusion. The agglutination reaction with *Streptococcus hemolyticus* and rheumatoid arthritis sera differs from that which occurs in certain febrile human sera in respect to its thermal characteristics. The agglutinating property in rheumatoid arthritis sera withstands heating at 55°C. and reacts with heat-killed suspensions.

6376

Studies in Venous Pressure. III. Effect of Gradually Increased Compression of Neck on Curve of Venous Pressure in Occluded Veins.*

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Eyster,[1, 4] Villaret, Girons, Bosveil,[2] Clark,[3] Eyster and Middleton,[5] and Lemierre and Bernard[6] among others have noted the elevation of venous pressure in congestive heart failure, which they report is of serious prognostic significance when it persists. Eyster[1, 4] concluded that an elevated venous pressure may point to impending cardiac decompensation, and that a sustained rise of general venous pressure is met only in this condition. More recent work has shown that other factors affect the level of venous pressure in man. Brandt[7] points out that the arteriolar pressure, the tonus of the veins, the visceral pressure in the abdomen and, most important of all, the circulating blood volume, also affect venous pressure. He found occasional coexistence of congestive heart failure and normal venous pressure, particularly in patients with auricular fibrillation or emphysema. Kroetz[8] believes that venous pressure may be affected by capillary and arterial pressure and by the venopressor mechanism described by Henderson.[9] Fleisch[10] suggests the possibility of a venomotor center in the brain. Brandt and Katz[11] found venous and arterial hypertension to coexist in the same patient, apparently due to an increased venous tonus. Arnoldi,[12] and Rotky and

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* Aided by the Emil and Fanny Wedeles Fund of Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

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[2] Villaret, M., Saint Girons, F., and Grellety-Bosveil, P., *Presse Med.*, 1923, **31**, 318.

[3] Clark, A. H., *Arch. Int. Med.*, 1915, **16**, 587.

[4] Eyster, J. A. E., *J. Am. Med. Assn.*, 1927, **89**, 428.

[5] Eyster, J. A. E., and Middleton, W. S., *Am. J. Med. Sci.*, 1927, **174**, 486.

[6] Lemierre, A., and Bernard, E., *Presse Med.*, 1926, **34**, 705.

[7] Brandt, F., *Ztschr. f. Klin. Med.*, 1931, **116**, 398.

[8] Kroetz, C., *Deut. Arch. f. Klin. Med.*, 1922, **139**, 325.

[9] Henderson, Y., *J. Am. Med. Assn.*, 1931, **97**, 1265.

[10] Fleisch, A., *Arch. f. d. ges Physiol.*, 1930, **225**, 26.

[11] Brandt, F., and Katz, G., *Z. f. d. ges Exp. Med.*, 1931, **76**, 158.

[12] Arnoldi, W., *Deut. Med. Wchnschr.*, 1920, **46**, 4.

Klein[13] found many cases of essential hypertension without evidence of cardiac failure with elevated venous pressure. Connet[14] found that merely slowing the normal heart may elevate venous pressure. Von Gonczy and Kiss[15] frequently found the venous pressure elevated during the menopause.

This study was undertaken to investigate further the correlation of the various factors controlling venous pressure. Dogs were used, under morphine and sodium barbital anesthesia. A tracheotomy tube was inserted just above the sternum; the external jugular vein and both carotids were exposed. Heparin was injected intravenously to prevent clotting. The external jugular vein was then severed high up in the neck and the horizontal arms of a short T tube inserted in each end. The perpendicular arm of the T tube was connected to an inflating rubber bulb and to a mercury manometer. A rubber cuff was then placed loosely between the T tube in the jugular vein and the tracheotomy tube, enclosing all the structures in the neck. Care was taken not to compress the jugular vein at this stage (checked by the venous pressure cephalad to the cuff).

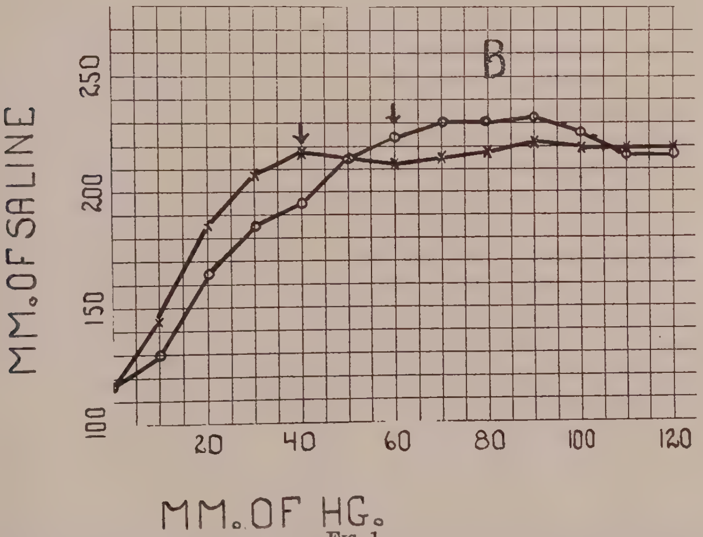

FIG. 1.

Curves of venous pressure of jugular vein following compression of neck. O—with carotid arteries exteriorized; X—with carotid arteries under compressing band.

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[13] Rotky, H., and Klein, O., *Med. Klin.*, 1923, **19**, 1542.

[14] Connet, H., *Am. J. Physiol.*, 1920, **54**, 96.

[15] von Gonczy, V. I., and Kiss, J., *Z. f. d. ges Exp. Med.*, 1931, **78**, 398.

The control level of venous pressure in the jugular vein having been determined, the pressure in the rubber cuff was then increased at intervals of 10 mm. of mercury at a time. The venous pressure was read off as millimeters of saline, after the level had become stabilized. The same animals were then used in similar experiments but with both common carotids exteriorized (the section of cuff under these vessels was clamped off in order to prevent the possibility of kinking or deforming the carotids during its inflation). In a few experiments both vertebral arteries were ligated.

The pressure noted in the graph (Fig. 1), while measuring the pressure within the rubber cuff, probably does not represent the degree of actual compression of the neck. The cuff was comparatively narrow and the overlapping ends were not parallel to each other, to avoid the protruding tracheotomy tube. These factors interfered with the full force of compression as registered by the mercury manometer. Nevertheless, the arrangement did provide a means of producing gradually increasing pressure around the neck.

A study of the curves so obtained shows that the venous pressure rises at an almost constant rate with increasing compression of the neck until a certain point is reached. Further compression of the neck results in little or no further rise, *viz.*, B of Fig. 1.

The point at which the curve changed from an oblique to a nearly horizontal line did not occur at the same stage of compression in different animals. In the entire series of 17, the change in slope usually occurred when the pressure within the constricting collar registered between 30 and 60 mm. of mercury, but there were a number of exceptions. In one dog the change occurred at 180 mm. of mercury. The proximity of the external jugular vein to the constricting cuff was the chief cause of this variation. Only 30 mm. of mercury pressure was necessary when the jugular vein was widely exposed and in actual contact with the cuff.

The level of venous pressure at which the pressure curve changed its slope also varied in different animals. In 9 animals the point was reached when the venous pressure was between 125 and 155 mm. of saline; in 6 at 200 and 250 mm. of saline; and in 2 at 370 and 515 mm. of saline.

Similar curves of venous pressure were obtained on gradually increasing the compression of the neck when both carotids were exteriorized, and when both vertebrals were ligated.

The results show that gradually increasing compression of the neck, under the experimental conditions described, produced a uniform type of change in venous pressure. The curve at first showed a rise of almost constant slope and after a certain point rapidly became less steep—practically horizontal—during further compression. Neither the degree of compression as registered in these experiments nor the height of the resulting venous pressure formed definite criteria for the level at which the curve changed in direction; nor was the degree of patency of the carotids and vertebrals an important factor. The one important variable was the accessibility of the vein.

The pressure within the external jugular vein is normally due to a number of factors, as outlined in the introduction. These factors may be divided into 3 groups: (a) the force of the heart beat as transmitted through the capillaries, (b) the tonus of the veins and, (c) the volume of blood within the veins studied. During gradually increasing compression the veins are probably the first to become closed, since the venous pressure is much lower than either capillary or arterial. When the veins are completely obliterated the inflow through the capillaries is still maintained, though possibly to a reduced degree, with the result that the veins continue to be filled. As the venous pressure rises, however, more and more resistance is offered to filling until a point is reached where the venous pressure practically equals the pressure in the capillaries, so that little, if any, additional filling can occur. The pressure rise thereafter will be less steep. This corresponds to B of Fig. 1. The eventual occlusion of the capillaries, probably soon after, acts in the same way.

No studies were made on the effect of venous stagnation in the head during these experiments on the CO_2 content of the blood. But an accumulation of CO_2 in the stagnant blood might be partly responsible for the contour of the curve, by acting on the brain or locally on the external jugular vein.

Summary. 1. A series of experiments was performed in dogs to determine the effect of gradually increased compression of the neck on the venous pressure of the occluded veins. 2. When the compressing pressure is plotted against the resulting rise in venous pressure the curve shows, at first, a steep slope up to a certain point. Further compression produces an abrupt change in slope and the assumption of a more or less horizontal level regardless of the further degree of compression.

We are indebted to Messrs. L. Kohn and I. Lauman for technical assistance in carrying out these experiments.

6377

Blood Plasma Volume Determination by Injection of Anti-Crystalline-Egg-Albumin-Serum.

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The blood plasma volume of an animal is usually found by determining the dilution in the plasma of a known amount of an injected test substance following its introduction into the blood stream. Dyes and other chemical substances are generally employed. The dilution of the material in the plasma is found (1) colorimetrically,[1, 2] (2) spectroscopically,[3] or (3) by chemical means.[4] There is a considerable variation in the figures obtained for blood plasma volume by these methods. This variation may be due partly to loss of the injected foreign material from the blood stream and partly to the difficulty of determining the dilution of the substance introduced. The injection of an immunologically distinct blood or serum would, presumably, be more harmonious with the blood and tissues of an experimental animal than a foreign dye or other chemical substance. The dilution of such a blood could be determined by means of immunological tests. This has been resorted to by several investigators for the determination of blood volume. The first to present this method was von Behring.[5] His procedure consisted in finding the dilution of a known amount of an antitoxic serum after its introduction into the blood stream. Later, Todd and White[6] employed isohemolysins to determine the dilution of blood from another animal of the same species after its injection. Ashby[7] described a similar method which made use of isohemagglutinins for finding the blood volume of man.

These immunological methods have not been widely used, although they would seem potentially advantageous since a native

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[1] Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, **16**, 547.

[2] Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **51**, 205.

[3] Graff, S., and Clark, H. T., *Arch. Int. Med.*, 1931, **48**, 808.

[4] Meek, W. J., and Gasser, H. S., *Am. J. Physiol.*, 1918, **47**, 302.

[5] von Behring, E., *Münch. med. Woch.*, 1911, **58**, 655.

[6] Todd, C., and White, R. G., *Proc. Roy. Soc. Lond.*, 1912, **84**B, 255.

[7] Ashby, W., *Med. Clinics North Am.*, 1920, **3**, 783; *Arch. Int. Med.*, 1925, **35**, 516, 632.

animal protein is administered. This is in part perhaps because the immune reactions concerned seem to lack sufficient accuracy for determining the dilution of the injected blood. We[8] have recently described a method for the quantitative estimation of the milligrams of precipitin antibody in rabbit anti-crystalline-egg-albumin-serum which may satisfy this requirement. The work reported here is an application of this method to the determination of the blood plasma volume of rabbits.

Method. A known volume of a precipitin-rich rabbit anti-crystalline-egg-albumin-serum is injected into the marginal ear vein of a normal rabbit immediately after the same amount of blood has been drawn from the recipient's heart. At intervals of 5, 15, and 60 minutes after the injection, 3 cc. blood samples are taken from an ear artery of the injected animal. By means of the neutralization method”[8] for estimating precipitins, the mg. of precipitin per cc. is determined for the injected serum and for the serum of the blood samples taken from the recipient rabbit after it received the antiserum. The plasma volume of the injected rabbit is found by the formula:

$$\text{Blood plasma volume} = \frac{\text{Mg. precipitin per cc. of injected antiserum x cc. of antiserum injected}}{\text{Mg. precipitin per cc. of recipient's serum post injection}}$$

TABLE I.
Data on Blood Plasma Volume Determinations.

Sex	Weight	Antiserum Injected	Precipitin Injected	Precipitin (Mg. per cc.)			Plasma vol. (15 min.)	Plasma vol. (15 min.) / Wt. of rabbit
				5 min.	15 min.	60 min.		
	gm.	cc.	mg. per cc.					%
M	2080	15	0.90	.213	.213	.189	63.0 cc.	3.1
M	2130	10	1.00	.119	.119	.119	82.0 cc.	3.8
M	2230	15	1.30	.213	.236	.213	82.5 cc.	3.7
M	2480	15	0.47	—	.084	—	82.5 cc.	3.3
M	2640	15	1.04	.189	.189	.143	82.5 cc.	3.1
F	1760	12	1.00	.166	.166	.143	72.0 cc.	4.0
F	2070	15	0.52	.094	.119	.094	64.5 cc.	3.1
F	3040	15	0.61	.107	.107	.107	85.5 cc.	2.8

The plasma volume of the 8 animals in this series is found to average 3.4% (that is, about one-twenty-ninth) of the body weight. The precipitin content of the 3 blood samples taken at intervals from each animal after the injection of the precipitating antiserum shows that the mixture of the antiserum and the blood of the injected animal is complete within 5 minutes and that the injected precipitin is retained quantitatively in the blood stream for at least 15 minutes after its introduction. The method here described seems to be a reasonable procedure for blood plasma volume determination and to possess certain advantages over other methods. A more extensive report of this work will be made elsewhere.

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[8] Culbertson, J. T., *J. Immunol.*, in press.

6378

Tobacco Sensitiveness in Thrombo-Angiitis Obliterans.

JOSEPH HARKAVY, SELIAN HEBALD AND SAMUEL SILBERT.
(Introduced by George Baehr.)

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The etiology of thrombo-angiitis obliterans is unknown. The current theories as to the cause are specific infections, endocrine disturbances, toxic factors, such as ergotism and tobacco. Thus far there is no decisive evidence in favor of specific infection. The rôle of tobacco has been stressed by Buerger, Erb, Meyer, L. F. Barker, Silbert and others. Statistically Barker has shown that the excessive use of tobacco is much more common in patients with thrombo-angiitis than in a control group of similar ages. The disease has been reported in individuals who do not use any tobacco. In the large experience of Silbert, however, no typical instances of thrombo-angiitis obliterans have ever been observed in non-smokers. Silbert has noted clinically that cessation in the use of tobacco has often definitely arrested the progress of the disease. While it is a fact that patients suffering from thrombo-angiitis are almost universally excessive smokers, it is also true that many equally extensive users of tobacco never develop thrombo-angiitis obliterans. If, therefore, tobacco plays an etiologic rôle, the question arises why this agent should affect one group of individuals, and not all others.

It occurred to one of us (J. H.) to investigate this problem from the point of view of specific hypersensitiveness to tobacco. Typical cases of thrombo-angiitis observed for a number of years in the Thrombo-angiitis Clinic were selected for investigation. This report gives the results obtained in 68 cases of thrombo-angiitis varying between the ages of 24 and 56, and 122 controls. All the control cases were males addicted to the use of tobacco, 47 healthy college students between the ages of 16 and 21, and 75 adults, chiefly Russian Jews, between the ages of 26 and 68. In this control group are 15 cases, suffering from circulatory disturbances of their extremities, atherosclerotic in nature; 5 of infectious asthma, and 15 cases of hay fever. These 75 controls were comparable in race, in the duration and amount of tobacco used, with the thrombo-angiitis patients.

Extracts of tobacco were prepared after the method of Coca. The tobacco used consisted of the uncured leaf furnished by the American Tobacco Co. Separate extracts were made from the following varieties: Burley, Maryland, Virginia and Xanthis (Turkish). A tobacco extract representing a mixture of various tobaccos obtained from the Allergy Department of the New York Hospital through the courtesy of Dr. Cooke, which we labelled "Coca" extract, was also employed. Five different extracts were tested on every case of thrombo-angiitis obliterans and every control. All the extracts were diluted 1-10, because the original solution was irritating and produced false positives. The technique used was the one employed in the testing of hay fever or asthma patients. The outer arm was injected intracutaneously with approximately 0.01 cc. of the tobacco extract and reactions read within 15-20 minutes. The results were as follows:

Out of 68 cases of thrombo-angiitis 58 or 83% reacted to one or another tobacco extract. Ten or 17% were negative. The reactions just as in other allergics varied in intensity. "Coca" extract which was a mixture of tobaccos naturally gave the largest number of positives.

Of 107 controls, all cigarette smokers, 97 were negative and 10 reacted to tobacco. or about 10%. In none of the 10% positives were the reactions marked, being at the most 2+. Analysis of this control group indicates that of the 47 college students 5 or about 10% reacted positively. Of the 55 individuals between the ages of 26 and 68, 5 also reacted. Fifteen patients included in this group who suffered from circulatory disturbances in their lower extremities of an arteriosclerotic nature were negative, although all of them were excessive cigarette smokers.

The five cases of infectious asthma were negative. A. Brown reports that 1% of asthmatics react to tobacco. All of the 15 cases of ragweed hay fever, however, reacted positively to tobacco. Whether these reactions were merely indications of the multiple sensitization observed in atopic individuals, or whether these patients were also tobacco sensitive, must be investigated further.

In contrast to this observation is the fact that only 2 out of the 68 thrombo-angiitis patients reacted to ragweed. One of these was a ragweed hayfever patient and the other had a father who was ragweed sensitive although he himself was free of symptoms. All of the other 66 were negative. They gave no atopic history.

A search for sensitization to substances other than ragweed and tobacco in 15 cases of thrombo-angiitis revealed definite reactions to foods and other inhalants. Thus 10 patients reacted to horse epithelium; 9 to tomato; 6 to rice; 5 to halibut; 4 to almond, beef, kapok, and cabbage; 3 to oats; 2 to cottonseed, lima bean and ragweed; one each to chicken, orange, eggwhite, lamb, black pepper, and pyrethrum.

While it is interesting to note that thrombo-angiitis patients, like allergic patients in general, also show multiple sensitization, no other antigen seems to be as dominant as tobacco. The importance of the simultaneous sensitization to foods, etc., however, is not to be disregarded. Further study may reveal some collateral significance.

Study of the character of the reactions to tobacco was made in 20 of the positively reacting patients with thrombo-angiitis to determine whether their reactions were dependent upon the presence of atopic reagins. The passive transfer technique of Prausnitz and Küstner was employed. Ten cc. of blood was drawn from the arm vein, clotted, and 0.1 cc. of the serum injected into various sites of the forearm of normal individuals. Three days later these sites were reinjected with the tobacco to which the patient had originally reacted. Out of 20 sera thus tested 13 gave positive passive transfers. The presence of reagins to tobacco in these thrombo-angiitis cases indicates that we are dealing with individuals who were in all probability atopic, and that the positive phenomena are true antigen-antibody reactions.

Summary. 1. 58 out of 68 cases of thrombo-angiitis or 83% were found to be hypersensitive to tobacco by means of positive skin reactions to various tobacco extracts. 2. The presence of atopic reagins to tobacco was demonstrated in 13 out of 20 patients by means of passive transfer. 3. Multiple sensitization characteristically prevalent in allergic individuals was also found in thrombo-angiitis patients. 4. Out of 102 control smokers only 10, or about 10%, reacted to tobacco. These reactions were at most 2+. In this group 15 cases with circulatory disturbances on an atherosclerotic basis were completely negative. 5. Ragweed patients usually reacted to tobacco, whereas only 2 patients in the entire group of 68 thrombo-angiitis sufferers reacted to ragweed.

Final conclusions cannot be drawn. Further investigation of other diseases for the occurrence of tobacco hypersensitiveness must be carried out before its importance in thrombo-angiitis can be definitely established. It has been clearly demonstrated, however, that a large percentage of patients suffering from thrombo-angiitis obliterans belong to the category of allergic individuals and that this allergy is essentially characterized by a hypersensitiveness to tobacco.

6379

Oral Immunization of Rabbits Against Pneumococcus Pneumonia and Septicemia.*

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As shown by Ross[1] it is possible to actively immunize rats against Type I pneumococcus septicemia by the oral administration of tissues carrying this organism as well as by feeding acid-killed, bile dissolved and mechanically disrupted organisms and the specific polysaccharide. Kolmer and Amano[2] found that rabbits could be sometimes immunized against highly fatal pneumococcus meningitis and septicemia, especially that produced by Type I pneumococcus, by the oral administration of heat killed and living vaccines. We have thought it worth while to ascertain if rabbits could be vaccinated against the pneumococcus pneumonia and septicemia induced by the intratracheal injection of living cultures as employed by Cecil and Steffen[3] in the production of pneumococcus pneumonia of monkeys. Cecil[4] reported that 3 subcutaneous injections of Type I pneumococcus vaccine protected these animals against pneumonia when inoculated intratracheally 3 weeks later.

Since it may be that the oral administration of pneumococcus vaccines may engender an equal or even higher degree of immunity

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* Aided by a grant from the Samuel F. Fels Fund for Medical Research.

[1] Ross, V., *J. Exp. Med.*, 1930, **51**, 585; *J. Immunol.*, 1926, **12**, 219, 237; *J. Lab. and Clin. Med.*, 1927, **12**, 566; PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 273.

[2] Kolmer, J. A., and Amano, K. W., *Archiv. Otolaryngol.*, 1932, **15**, 547.

[3] Cecil, R. L., and Steffen, G. I., *J. Exp. Med.*, 1921, **34**, 245; 1923, **38**, 149.

[4] Cecil, R. L., *Arch. Int. Med.*, 1928, **41**, 295.

than subcutaneous injections we thought it advisable to start with the highly susceptible rabbit and to continue the experiments with monkeys in case encouraging results were obtained. Three kinds of vaccines were employed after the methods developed by Ross. That designated as "milk-heated" was prepared by cultivating the organisms in sterile milk for 24 hours and heating at 60°C. for an hour. That designated as "acid-killed" was prepared by cultivating the pneumococci in a broth medium for 24 hours and adding sufficient N/1 hydrochloric acid to give a N/15 concentration. After standing for 2 hours at room temperature the vaccines were subcultured for sterility. That designated as "bile-dissolved" was prepared as follows: Broth cultures were cultivated for 24 hours at 37°C. They were then thoroughly centrifuged and the cocci from each 50 cc. dissolved in 5 cc. of a 1% solution of sodium taurocholate. Medium sized rabbits were given 7 daily doses of each vaccine by stomach tube. The dose of the milk-heated and acid-killed vaccines was 10 cc. per animal or approximately 5 cc. per kilo of weight while the bile-dissolved vaccine was given in dose of 5 cc. per animal or approximately 2 cc. per kilo. One week after the last or seventh dose each animal was injected intratracheally† with sufficient amounts of living broth cultures to produce the pneumonia and septicemia characteristic of these animals. With our Type I pneumococcus the intratracheal injection of 0.5 cc. of a 1:30 dilution of 6 to 8 hour broth culture invariably produced a fatal pneumonia and septicemia (positive heart blood cultures) in 3 to 5 days. Our Type II strain, however, was much less virulent, as the injection of as much as 2 cc. of a 6 hour broth culture produced fatal pneumonia and septicemia in only about 50% of the unvaccinated controls. The same was true of the Type III strain, since the injection of 2.5 cc. of 6 hour broth culture produced fatal infections in about 40 to 50% of the controls.

We believe that the results definitely show that rabbits may be effectively immunized against virulent Types I, II and III pneumococci by the oral administration of these 3 kinds of pneumococcus vaccines. Particularly good results were observed with the Type I vaccines and especially the acid-killed and milk-heated ones. As shown in the results of an experiment summarized in Table I as an example of several, while all of 12 unvaccinated controls succumbed to the pneumonia and septicemia induced by intratracheal inoculation, from 33 to 75% of the immunized animals survived indefinitely.

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† All intratracheal inoculations were conducted under ether anesthesia.

TABLE I.

Oral Immunization of Rabbits Against Type I Pneumococcus Pneumonia and Septicemia. Intratracheal Inoculation.

Vaccines	No. Immunized	Survived	Died	% Survivals
Acid-killed	12	9	3	75
Bile-dissolved	6	2	4	33
Milk-heated	6	4	2	66
Controls (12)	0	0	12	0

Furthermore this induced or active immunity has been found to endure for at least 4 months and possibly longer. At present all of a series of 12 rabbits immunized 4 months ago with 7 daily doses of "acid-killed" and "milk-heated" vaccines survived intratracheal injections while all of a control series of 4 animals succumbed in the usual period of 2 to 5 days.

With the Type II vaccines the results have been less definite and convincing, since about half of the unvaccinated controls survived intratracheal inoculation. In one experiment summarized in Table II as an example of several, the "acid-killed" and "milk-heated" vaccines protected all of the vaccinated animals while the "bile dissolved" vaccine produced much less protection. In some experiments, however, the latter gave results similar to those observed with the 2 former vaccines so that we have reason to believe that Type II pneumococcus vaccines by oral administration are capable of engendering some degree of active immunity.

TABLE II.

Oral Immunization of Rabbits Against Type II Pneumococcus Pneumonia and Septicemia. Intratracheal Inoculation.

Vaccines	No. Immunized	Survived	Died	% Survivals
Acid-killed	12	12	0	100
Bile-dissolved	6	4	2	66
Milk-heated	6	6	0	100
Controls (10)	0	5	5	50

Similar results were observed with Type III vaccines, the results of one experiment being summarized in Table III as an example of several conducted with Type III pneumococcus. While from 40 to 60% of unvaccinated controls survived indefinitely because of the low virulence of our strain, yet the percentage of survivals among the immunized animals was appreciably higher as indicated by the results of the experiment summarized in Table III.

No experiments were conducted with pneumococci belonging to Group IV. But at present we are investigating results of immunization with smaller doses of vaccine, the effects of fewer doses, and further experiments upon the duration of the active immunity.

In general it would appear that the "acid-killed" vaccine has pro-

TABLE III.
Oral Immunization of Rabbits Against Type III Pneumococcus Pneumonia and Septicemia. Intratracheal Inoculation.

Vaccines	No. Immunized	Survived	Died	% Survivals
Acid-killed	12	100	0	100
Bile-dissolved	6	100	0	100
Milk-heated	6	4	2	66
Controls (10)	0	6	4	60

duced the most encouraging and consistent results and our present experiments are being conducted only with this particular kind. Furthermore, we believe that the results require further study employing monkeys, in view of the fact that the induced pneumonia bears a closer resemblance to pneumococcus lobar pneumonia of human beings as shown by Cecil and his associates.

Summary. 1. It has been found possible to actively immunize rabbits against Type I pneumococcus pneumonia and septicemia produced by the intratracheal injection of virulent culture, by the oral administration of 7 doses of vaccines at daily intervals. 2. Of the 3 "acid-killed", "bile-dissolved" and "milk-heated" vaccines employed, the first mentioned appeared to engender the most immunity. 3. The induced immunity has been found to persist for at least 4 months and possibly longer. 4. Oral administration of similar vaccines of Types II and III pneumococci also produced active immunity but probably not of a degree equal to that engendered by Type I pneumococcus.

We are greatly indebted to Mr. Samuel F. Fels for generous financial assistance in conducting this investigation.

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Nuclear Phenomena Suggesting a Sexual Mechanism for the Tubercle Bacillus.

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This inquiry takes immediate origin in the suggestive rôle of the macrotetrads in the life cycle of the avian tubercle bacillus (Mellon, *et al.*[1]); more remotely, in the proven rôle of macrotetrads in diphtheroidal-streptococcus transformations.[2]

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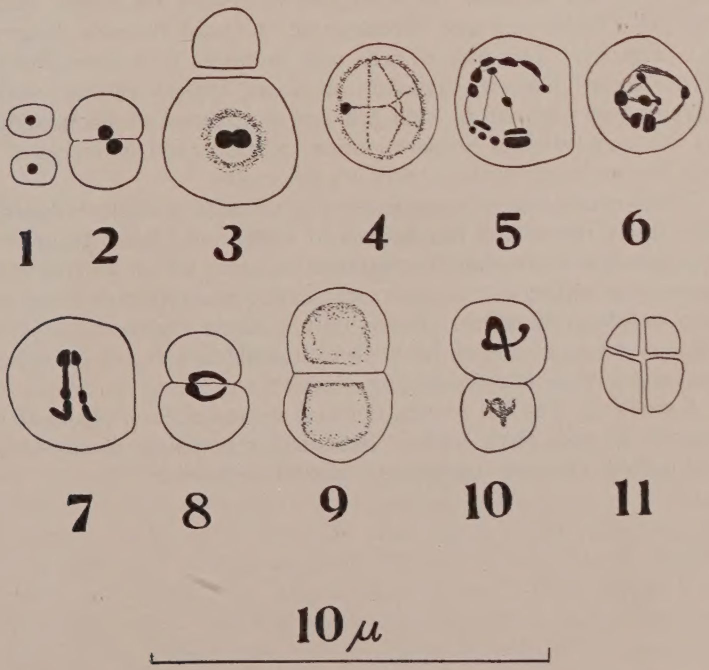
[1] Mellon, R. R., Richardson, R. D., and Fisher, L. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 80.

[2] Mellon, R. R., *J. Med. Res.*, 1920, **42**, 61.

Observations in the hanging drop have shown single macrotetrads, germinating as tiny cocci; which on agar slants again yield macrotetrads, thus completing a short cycle. Modern cytological technique has revealed nuclear phenomena of this cycle which are conventionally obscured. Figs. 1 to 11 are camera lucida reconstructions from aceto-carmine stained slides, and the sequence may not be valid. As against the possibility that the sequence indicated by the figures is incorrect there is the fact that this series (Figs. 4-11) resembles in a striking manner the stages of meiosis. Acknowledging the possibility of misinterpretation, the following hypothetical explanation is proposed.

Each coccus contains a single nucleus. These nuclei are about 0.2μ in diameter, and stain very heavily without showing vesicular structure. Two such cocci fuse by solution of a portion of their adjacent cell walls (Fig. 2) and one nucleus migrates into the adjoining coccus. This cell (Fig. 3) increases greatly in size. At one end the empty cell is still attached. In the larger cell the 2 nuclei fuse. This fusion takes place in a colorless area surrounded by the deeper staining cytoplasm. The zygote nucleus increases in



size until it nearly fills the cell and assumes the typical vesicular appearance of a true resting nucleus (Fig. 4). It has a definite membrane and contains many long, slender, faintly-staining threads. The increase in size following the sexual fusion parallels in a striking manner the nuclear phenomena in the young ascus of Neurospora and other Ascomycetes. Densely staining threads and granules appear in the nucleus. As many as 14 particles connected by slender threads have been found in a single cell (Fig. 5). These densely staining granules are the chromomeres. The number of chromomeres decreases and the zygote continues to shrink. Finally, about 7 discrete particles are visible in the cell (Fig. 6). Apparently about 7 chromomeres have been elaborated from each of the fusing nuclei. These have synapsed, producing 7 fused chromomeres. The chromomeres approach each other by the shortening of the intervening threads. Two chromosomes are produced (Fig. 7). One contains 4 nodes while the other contains 3 nodes. If this were a standard meiosis each chromosome should contain all 7 chromomeres. The morphology of these chromosomes is remarkably constant with respect to the 2 small terminal nodes and the thick and thin threads. It is difficult to resolve the larger nodes. The cell divides and one chromosome is found in each daughter cell (Fig. 8). This pair of cells now increases in volume and the chromosomes become vacuolate to form typical resting nuclei. Chromosomes appear in each cell and the second division occurs. As the tetrad divides on germination to form cocci, it increases in bulk, but no larger nuclei or cells are discernible.

These cytological observations have revealed a nuclear mechanism closely resembling the meiosis of fungi and higher forms. If hybridization is possible, the extreme variation which so frequently appears on plating out a culture containing macrotetrads is capable of a simple explanation. But if only a single chromosome exists, the only reassortment of factors which could be effected by hybridization would be due to crossing over.

Summary. Stained preparations of a non-acid-fast phase of the tubercle bacillus show nuclear structures resembling chromosomes, and nuclear changes suggesting a sexual mechanism.